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Examination	Requested
Title of Invention	The rubber-elasticity kinds is the biodegradation property poly -3-hydroxyalkanoate production cell group and polymer composition.

Abstract

C which cultivates in the culture medium including the unsaturated carboxylic acid and/or the saturation the Pseudomonas sp. HJ-2 strain deposited to the KCTC 0406 BP and produced.3~CC and/or the monomer of 5.6~CWhile the bio compatibility, and the biodegradation property and elasticity are excellent, it can control the elasticity according to the kind of the substrate carbon source, and concentration and condition of culture and the polyhydroxyalkanoate copolymer including the monomer of 14 can be usefully used in the agriculture, everyday supply, the medical instrument, food packaging and medical field.

Representative Drawing(s)

Fig. 1

Description

※ Brief Explanation of the Drawing(s)

Figure 1 is a graph of the cell growth about the elapsed time in the blanket supply fermentation and remaining substrate number of heptanoate by the Pseudomonas sp. HJ-2.

Figure 2 is a gas chromatogram of the sample which the edge methanol reacts and obtains the poly (3HB-co-3HV-co-3HHp) which synthesizes with the Pseudomonas sp. HJ-2 from heptanoate.

Figure 3 is a graph showing the change of the PHA composition about the elapsed time in the blanket supply fermentation of heptanoate by the Pseudomonas sp. HJ-2.

Figure 4 is a thermogram measuring the melting point of the poly (3HB-co-3HV-co-3HHp) synthesized with the Pseudomonas sp. HJ-2 from heptanoate.

※ Details of the Invention

※ Purpose of the Invention

※ The Technical Field to which the Invention belongs and the Prior Art in that Field

[Industrial applicability]

The invention relates to polyhydroxyalkanoate with a superior bio compatibility and biodegradation property, more specifically, to polyhydroxyalkanoate and the production cell group which is useful in all fields in which degradable and elasticity including the plumbing fixtures, which has the monomer the bio compatibility and biodegradation property are excellent, various as the various composition, freely can control the elasticity by doing, the medical appliance, the medical instrument etc. are required.

[Prior art]

The synthetic plastics and the synthetic fiber products widely used since convention due to the excellent durability for the daily life cause the serious environment contamination problem falling into disuse after use and nots being disassembled in the nature system even after the several tens year passes by polluting the soil and river and hindering the growth of plant, etc. It is in the excel durability of the plastic endured against the physicochemical change of the neighboring including the reason of the environmental contamination is the decomposition of microorganism by this kind of plastic.

In order to solve the environment contamination problem by this kind of plastic, the alive disintergrability plastic disassembled with microorganism was developed. It added the starch or the metal complex in the existing synthetic plastics including polyolefin or the polyester etc. and these alive disintergrability plastics manufactured. In that way it was manufactured in order to be easily collapsed with the work for including the decomposition and photooxidation of microorganism etc. But there is a problem that the analysis of starchy easily occurs under the plastic component but the synthetic plastics part is not still disassembled, it has and this kind of the alive disintergrability plastic pollutes environment. In addition, and, the other environmental pollution sources can become the used metal complex part in these plastic manufactures.

Recently, in order to fundamentally solve the environment contamination problem by this kind of synthetic plastics, the research about the bioplastic (bioplastics) generated from the creature itself actively proceeds. As the natural polymer material which these bioplastics called as the biopolymer are directly obtained from microorganism, and the animal or plant, it has the excellent mechanical durability and these middle a parts can be used for material including the general material, the packing material, the agriculfural film etc. like the existing synthetic plastics. In addition, it has the very excellent biodegradation property and if the fixed time passes, it is completely disassembled to the water and carbon dioxide in the nature system. And it has the bio

compatibility excellent with being harmless in the human body and it is used for material including the adhesive, used for organism band, the sealing fiber etc.

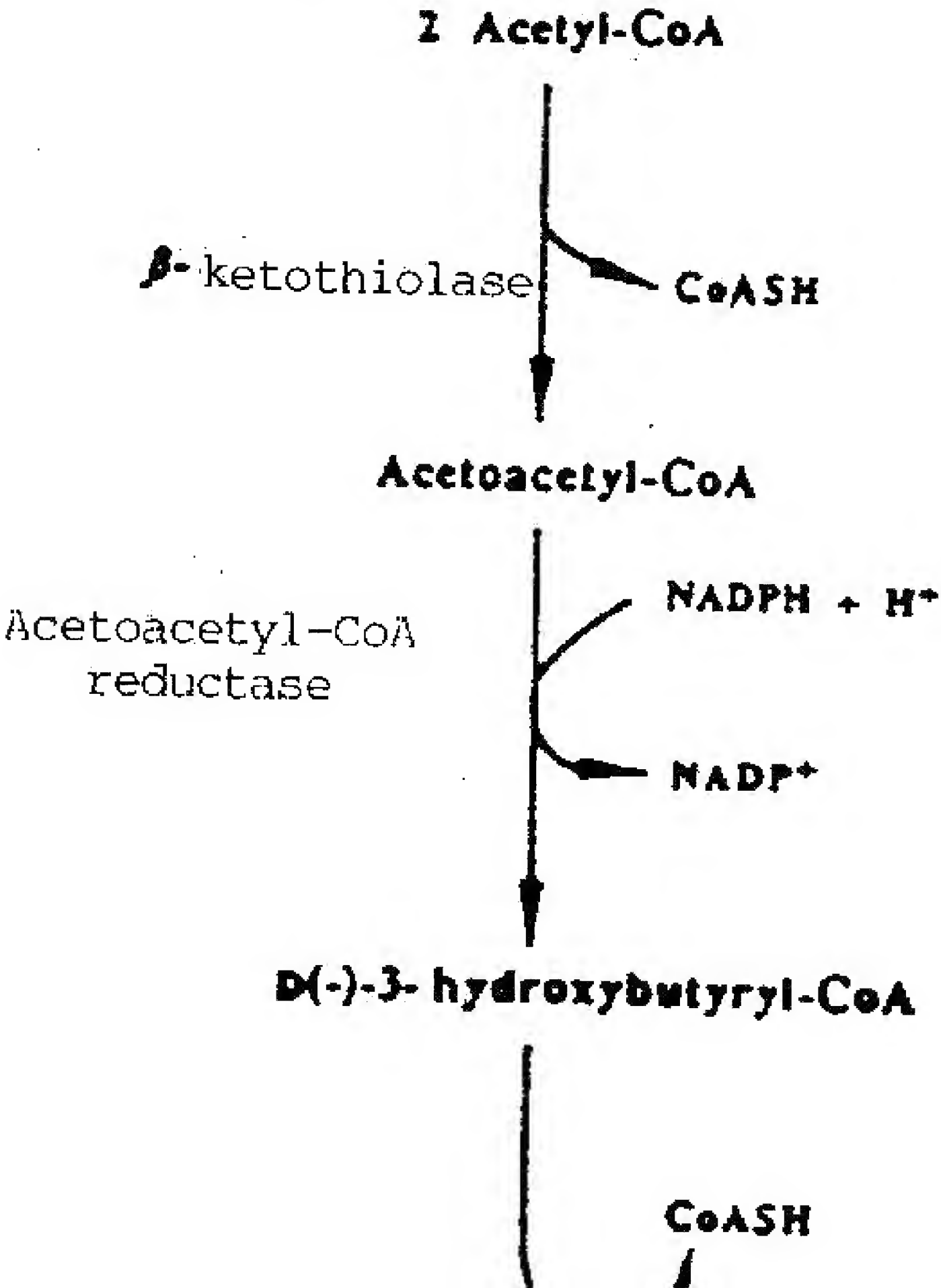
The industrial adaptation possibility of PHB having the midterm property of a kind of these middle poly-beta - hydroxyalkanoate (hereinafter the poly- β -hydroxyalkanoates: says to be the PHAs) the polyester and polypropylene it so far has *** coal (pullutan), chitosan, xanthan, gellan, acid (rhamsan), the poly-beta - hydroxybutyrate (hereinafter the poly- β -hydroxybutyrate says to be PHB) etc to the publicly known bioplastic are known to be most big. In this PHB is the nature system, it is completely disassembled with microorganism. And it has the property of the etc. naturally absorbed and decomposed in organism when using within organism. It is the creature thermoplastic plastic (biothermoplastics) which can completely solve the environment contamination problem.

Generally, the in this way many research that PHB which is one of Bacillus megaterium by the M. Lemoigne in 1926 year as the storage material, which as to PHA, a part bacteria stores to the carbon source and energy source for the first time, the PHAs is discovered (Poivier et al., Biotechnol. 13:142-150, 1995) proceeds. PHAs exist within cell in the form of the inclusion body. And the accumulation rate increases under the condition (unbalanced condition) of the unbalanced in which the nitrogen (N), the phosphorus (P) and sulfur (S) etc. are restricted and cell is known. The carbon number of 3- hydroxyalkanoate (3-hydroxyalkanoates), which PHAs are the monomer comprising PHAs a lot, the short-chain length (Short-Chain-Length, and the SCL: C.3~C5The) PHAs and the heavy chain length (Medium-Chain-Length, and the MCL: C.6~C14The chain-lengthened length (Long-Chain-Length, LCL) PHAs having without, monomer between 18 in 15 carbon numbers it is divided into the do-group of the) PHAs was reported (Song et al., J. Microbiol. Biotechnol. 3:123-128, 1993). The difference of this length is the affinity difference because of the PHA synthase about 3- hydroxy alkyl -CoA which is the precursor of the PHAs.

The biosynthesis bacteria was till now the discovered PHAs 90 inside or greater. The PHAs monomer of about 90 kind was discovered (Song et al., J. Microbiol. Biotechnol. 3:123-128, 1993). Among them, in the bacteria which the biosynthesis represents the short-chain length PHAs, it has the Alcaligenes sp. and Bacillus spp etc. The biosynthesis is the PHB homopolymer which is one of the short-chain length PHA increasing with especially, the Alcaligenes sp known as the representative bacteria.

The melting point of PHB the heavy chain road which is 40~60℃ is high as about 180℃ in comparision with PHA (Babu et al., International symposium on bacterial polyhydroxyalkanoates. 48-54, 1996). The route (pathway) of representing that the short-chain length PHA is synthesized is according to the Alcaligenes spp. The acetoacetyl-CoA is made, it is initiated in two acetylcoenzyme As (acetyl-CoA) and PHB is weighed with the work for of enzyme with the biosynthesis heartburnings (Poivier et al., Biotechnol. 13:142-150, 1995). The biosynthetic pathway of PHB by the Alcaligenes eutrophus was shown in the estival equation 1.

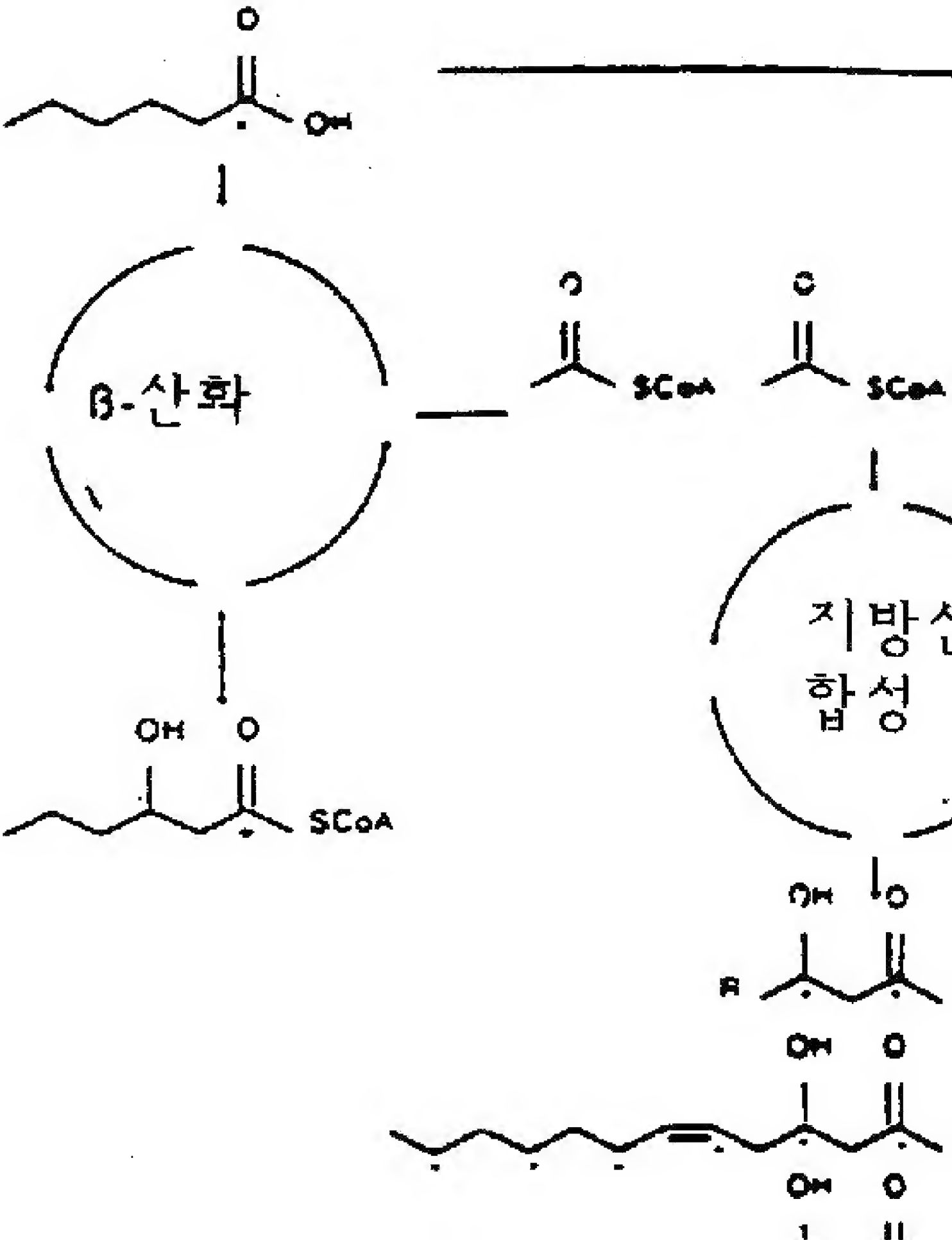
[Equation 1]



In the biosynthesis is the A. eutrophus PHB, the Chromobacterium violaceum the biosynthesis is the PHV homopolymer known from the valerate as thing while accumulating PHB to the maximum 80% of the gun body weight (Anderson et al., Microbiol. Rev. 54:450-472, 1990; Steinbuchel et al., FEMS Microbiol. Lett. 128:219-228, 1995). Moreover, in a part bacteria, when the propionate, valerate, and the cosubstrate like γ - hydroxy butyrolactone (γ-hydroxy butyrolactone) are together put besides the cycle quality and it cultivates, with the copolyester which is the same as that of the poly (3HB-co-3HV), and the poly (3HB-co-4HB) synthesizing it is known.

The research about the biosynthesis of PHA the heavy chain road is accomplished with present, mainly, the Pseudomonas oleovorans, and the Pseudomonas spp asser like the Pseudomonas putida, extensively. And the heavy chain road consisting of the short monomer which is longer than the substrate which is mainly given through the process of the de novo biosynthesis of fatty acids (denovo fatty acid biosynthesis) and β - oxidation (β-oxidation) or installs PHA with the biosynthesis heartburnings. The reaction obtaining the precursor for the PHA synthesis from hexanoate was shown for the estival equation 2. 3- hydroxyhexanoate monomer is synthesized after β - oxidation process. The acetylcoenzyme A molecule which at the same time, is generated is put into into PHA after the de novo biosynthesis of fatty acids.

[Equation 2]



The heavy chain road as to PHAs, has to low to melt. And is low to the short-chain length PHAs it has the property of the strong rubber elastic body as the length of the monomer side chain is lengthened (Babu et al., International symposium on bacterial polyhydroxyalkanoates. 48-54, 1996).

It has with the biosynthesis but the most of bacterias the biosynthesis is the short-chain length monomer and the PHAs in which monomer of the heavy chain road is altogether contained one of short-chain length PHAs or the heavy chain road is the PHAses known in a part Pseudomonas spp as thing (Kato et al., Appl. Microbiol Biotechnol. 45:363-370, 1996; Lee et al., Appl. Microbiol Biotechnol. 42:901-909, 1995; Steinbuchel et al., Appl. Microbiol Biotechnol. 37:691-697, 1992). Because of having the various property which it so far does not inform, the short-chain length monomer and PHA in which the heavy chain road altogether contains monomer receive many concern. Moreover, in the biosynthesis the biosynthesis, the heavy chain road the transformation (transformation) one reunion germ (recombinant strain) in the P. oleovorans the poly (3HB) the poly (3HB) - synthetic gene of the A. eutrophus the PHAs, the biosynthesis summer solstice these are the short-chain length monomer and the PHAs in which monomer together has the heavy chain road the physical mixture (Timm et al., Appl. Microbiol Biotechnol. 33:296-301, 1990).

◈ The Technical Challenges of the Invention

An object of the present invention are to provide PHA and the production cell group having the elasticity and the elasticity which desires by it makes monomer various, controlling the composition rate within PHA with a superior biodegradation property and bio compatibility.

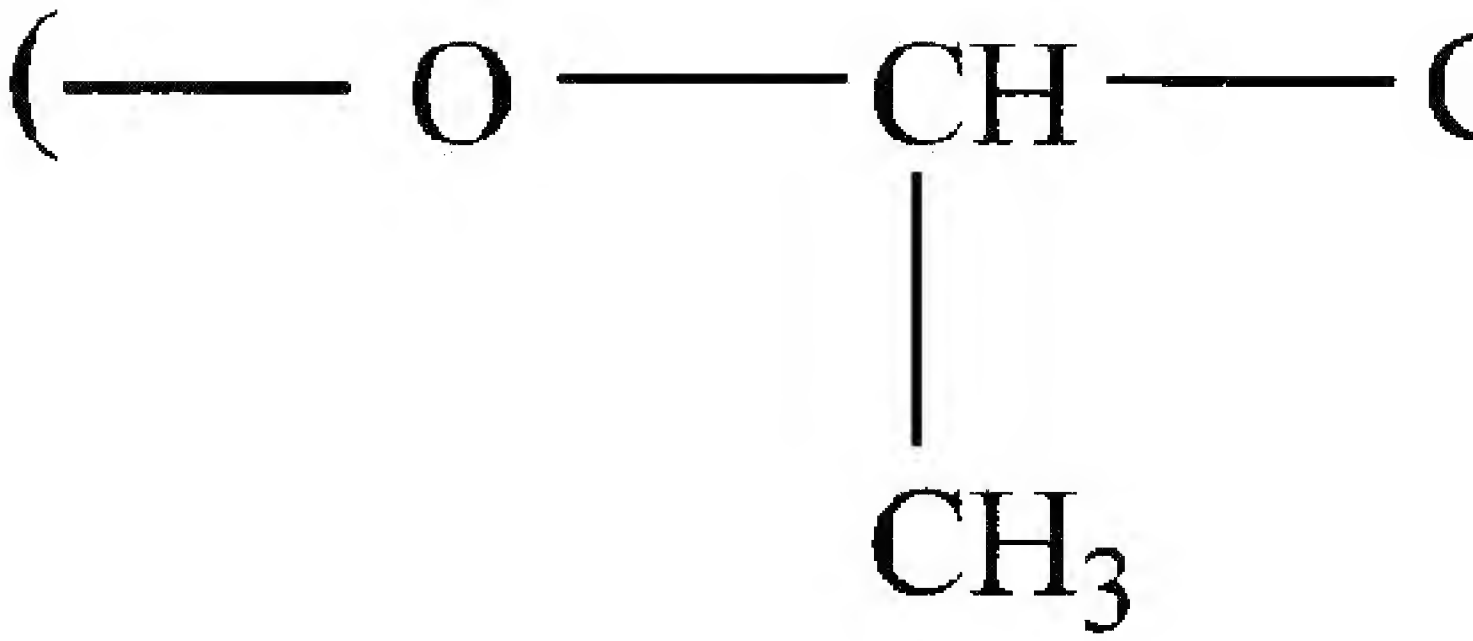
◙ Structure & Operation of the Invention

[The means for solving the subject]

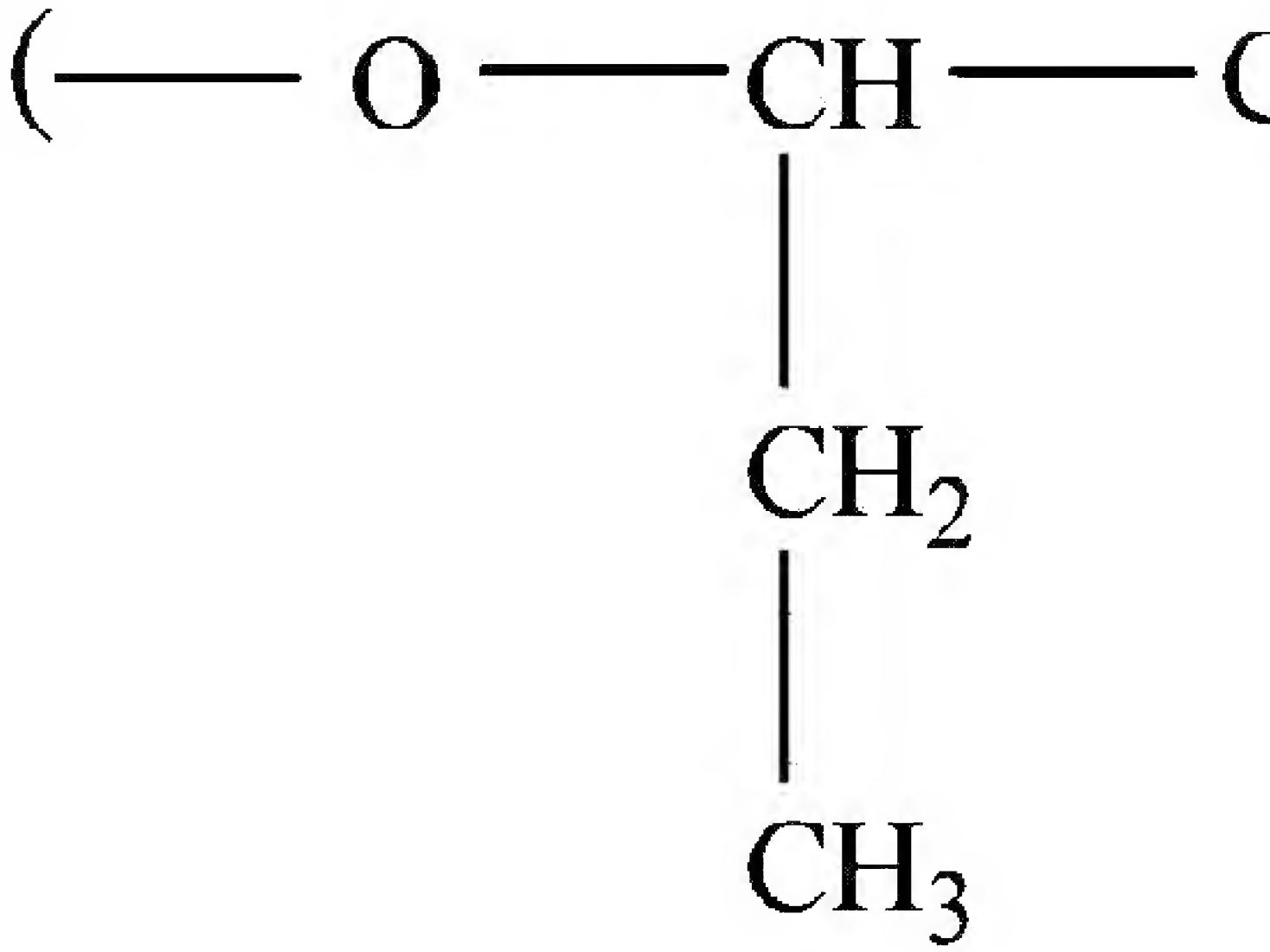
The invention as described above, to accomplish the above objects, the invention is C.4, C5, C6, C7, C8, C9, C10, C11, C12, C13And c14One selected from the group consisting of monomer or the Pseudomonas sp. HJ-2 strain which is the monomer described in the above deposited in Korea Institute of Science and Technology gene engineering center Center for Biotechnology Information Genbank producing the polyhydroxyalkanoate copolymer included as the main monomer to the KCTC 0406 BP is provided.

It is preferable that the above-described monomer is 3- hydroxybutyrate of the estival chemical formula 1, 3-hydroxy heptanoate of 3- hydroxyvalerate of the chemical formula 2 and chemical formula 3.

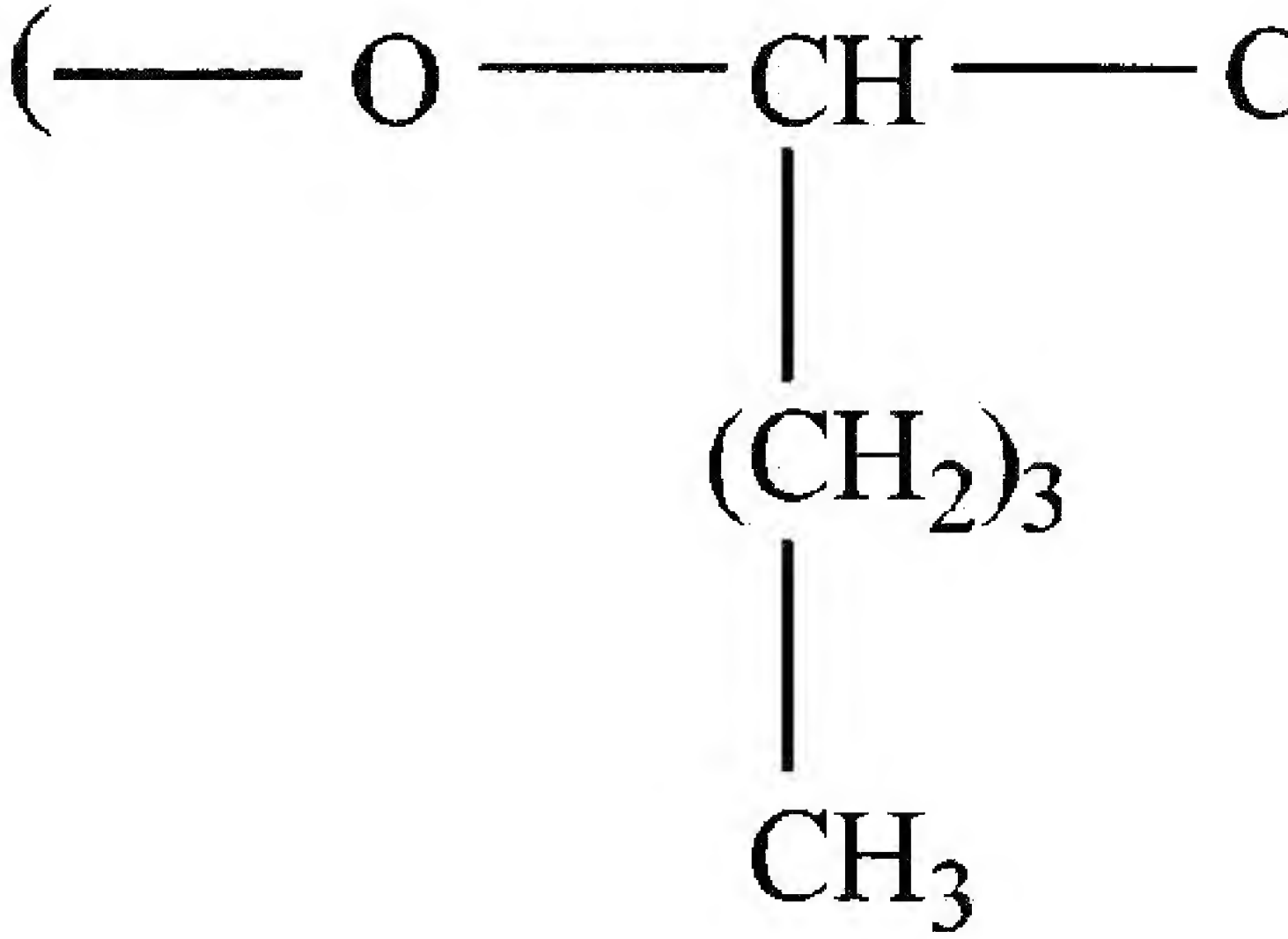
[Chemical formula 1]



[Chemical formula 2]



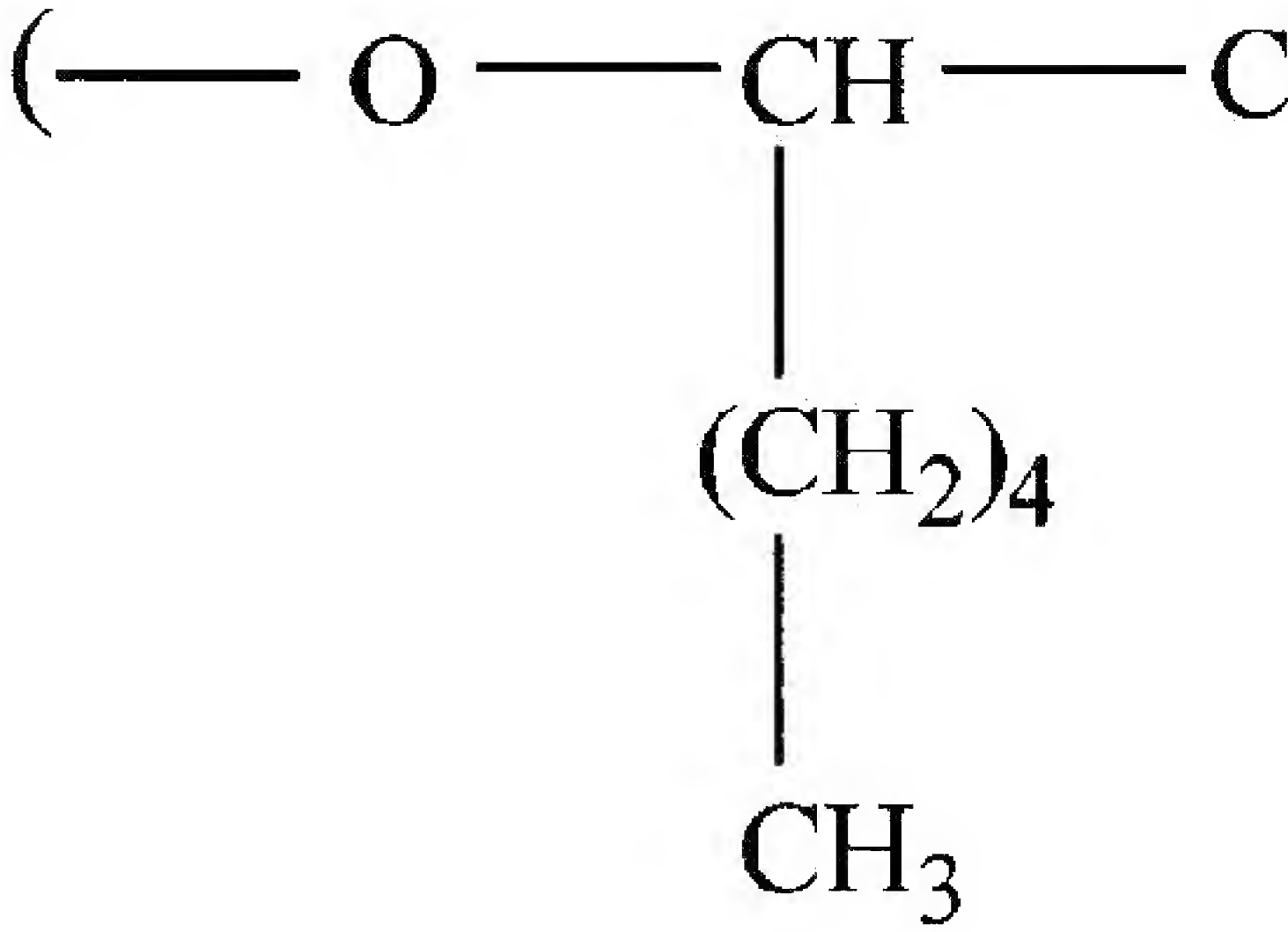
[Chemical formula 3]



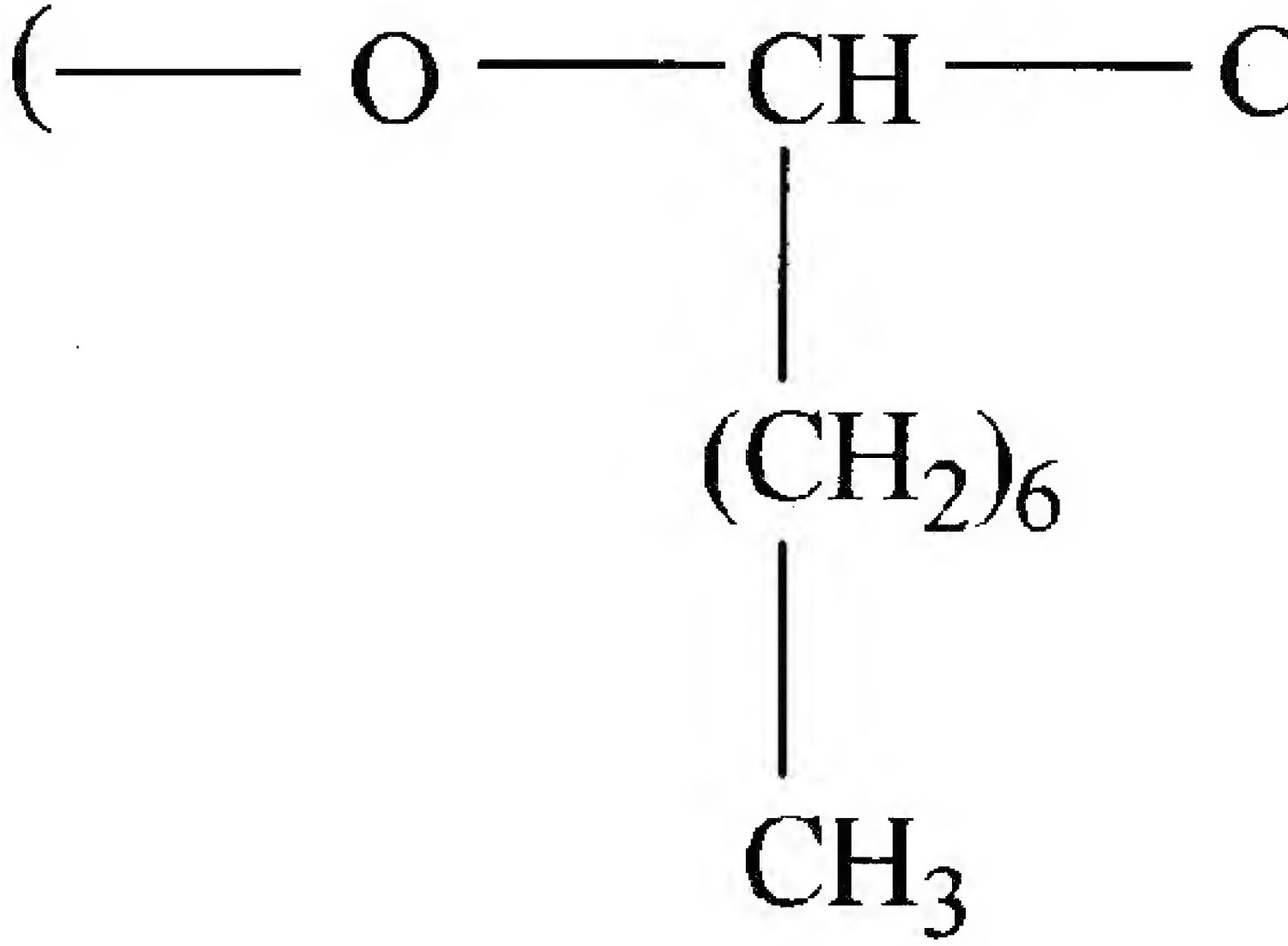
A of the chemical formula 1 5~100% , and the b of the chemical formula 2 are 0~95%. The c of the chemical formula 3 is 0~80%.

Moreover, it is preferable that it is 3- hydroxyoctanoate of the estival chemical formula 4, 3- hydroxy dodecanoate of 3- hydroxy decanoate of the chemical formula 5 and chemical formula 6.

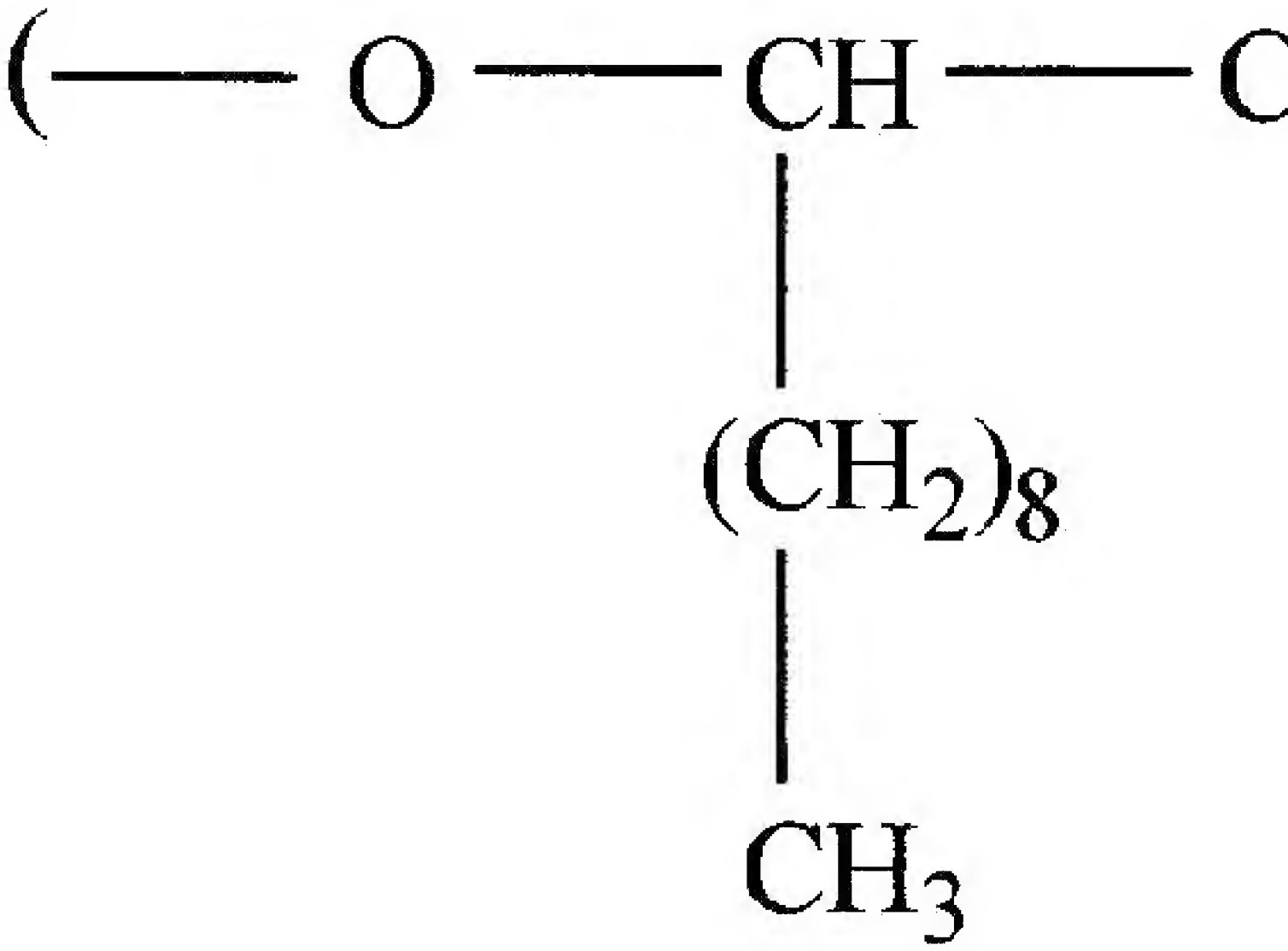
[Chemical formula 4]



[Chemical formula 5]



[Chemical formula 6]



The d of the chemical formula 4 described in the above 0~100% , and the e of the chemical formula 5 are 0~100%. The f of the chemical formula 6 is 0~100%.

It is preferable that the above-described strain the culture medium including the unsaturated carboxylic acid hydrolyzing the gasoline selected from the group consisting of the vegetable oil, and the animal fat and fish gasoline and is obtained and/or the saturation to the carbon source and it synthesizes polyhydroxyalkanoate.

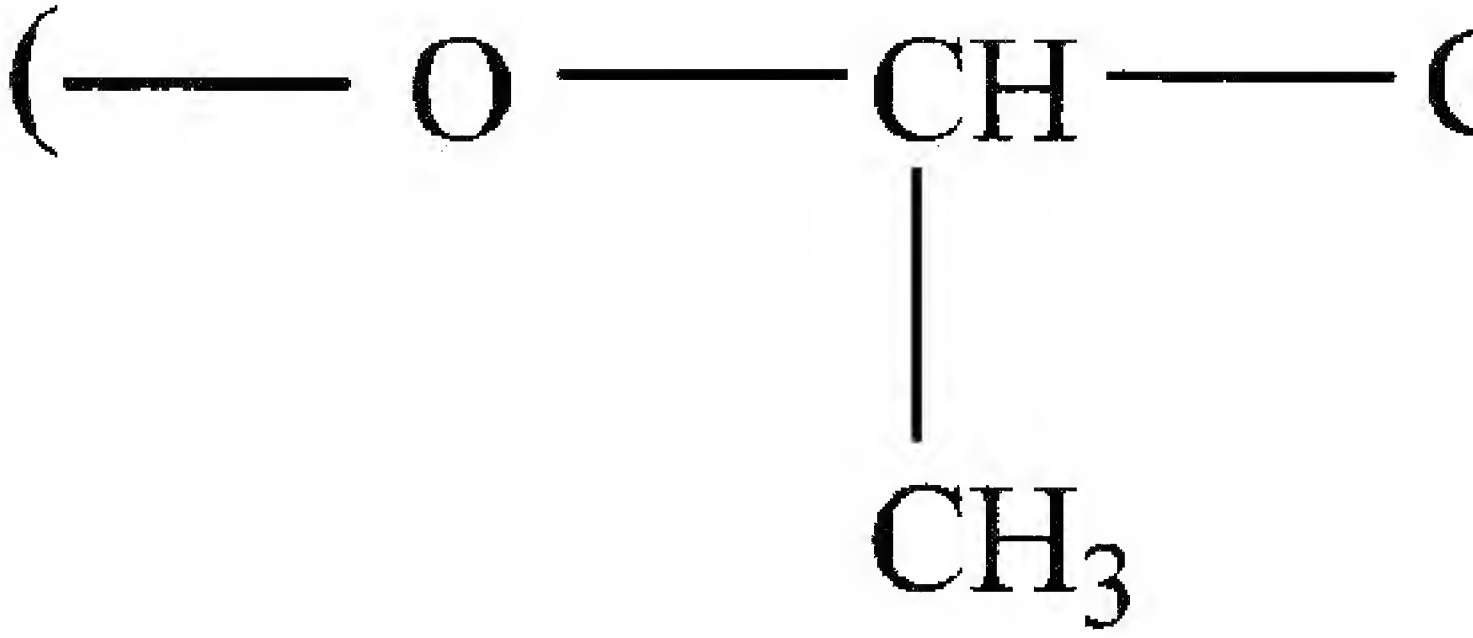
It is preferable that the equation property or the animal fat is selected in the group consisting of the cooking oil, the disposable edible oil, and the lard and tallow than.

It filters the solid which adds the NaOH until it is entangled like the soap while it maintains so that it adds the water according to need while mixing with the distilled water and heating and the water be mixed and obtained or it uses the gasoline including the cooking oil etc. as itself after the preprocessing dried.

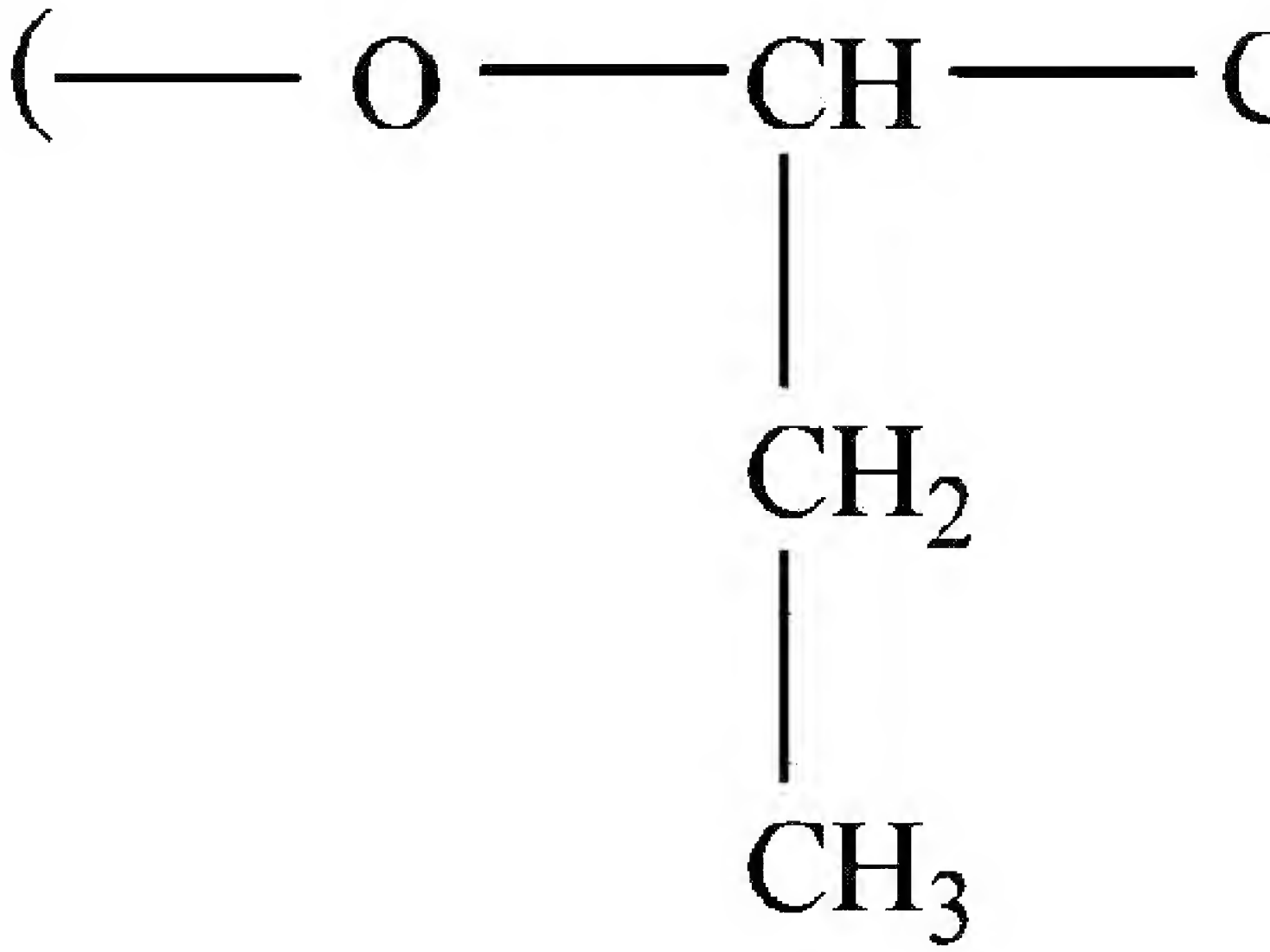
Moreover, in the present invention, c4, C5, C6, C7, C8, C9, C10,C11, C12, C13And c14Two selected from the group consisting of monomer or the polyhydroxyalkanoate copolymer including the monomer described in the above to the main monomer is provided.

It is preferable that the above-described monomer is the polyhydroxyalkanoate copolymer which is 3-hydroxybutyrate of the estival chemical formula 1, 3- hydroxy heptanoate of 3- hydroxyvalerate of the chemical formula 2 and chemical formula 3.

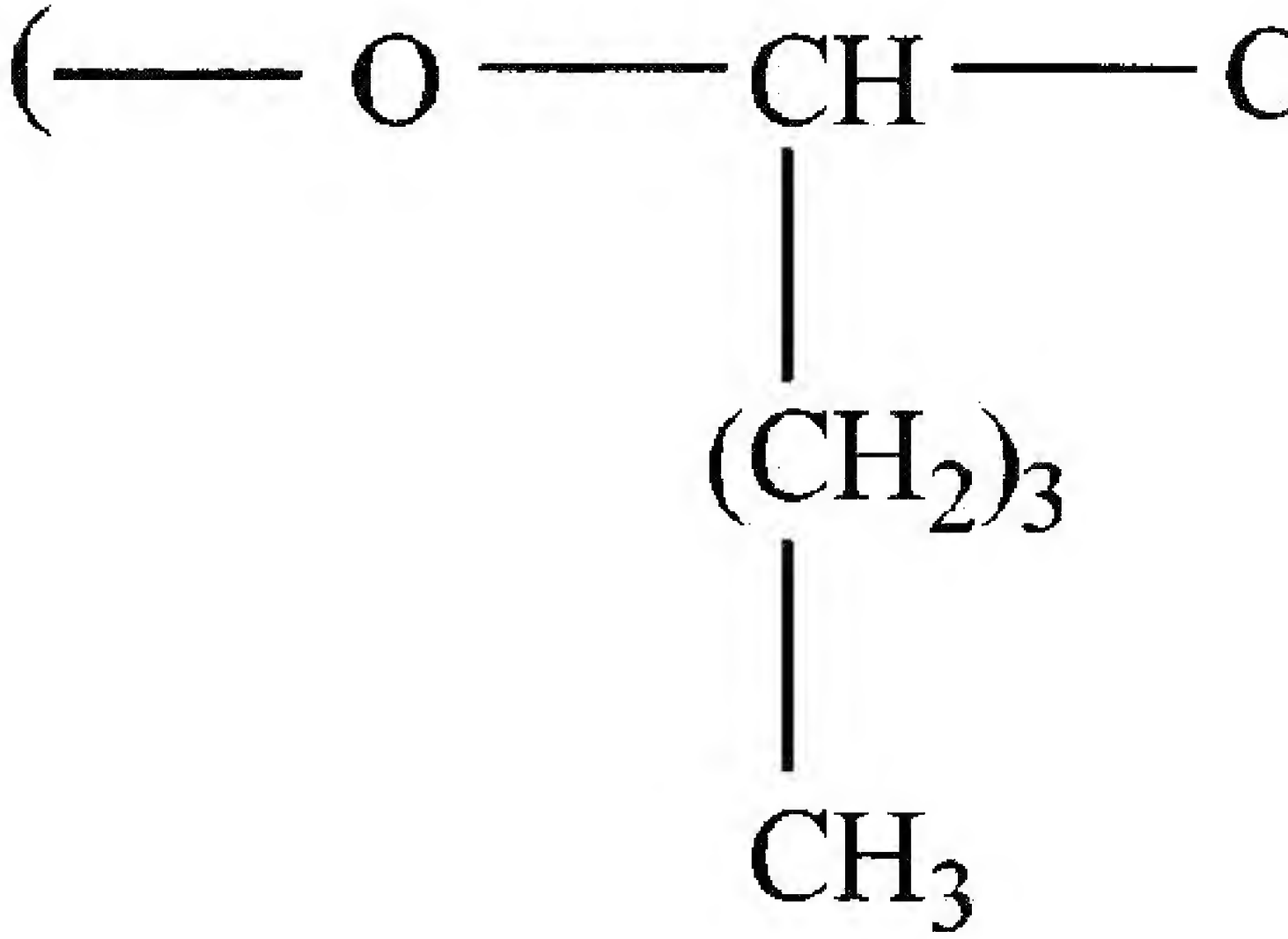
[Chemical formula 1]



[Chemical formula 2]



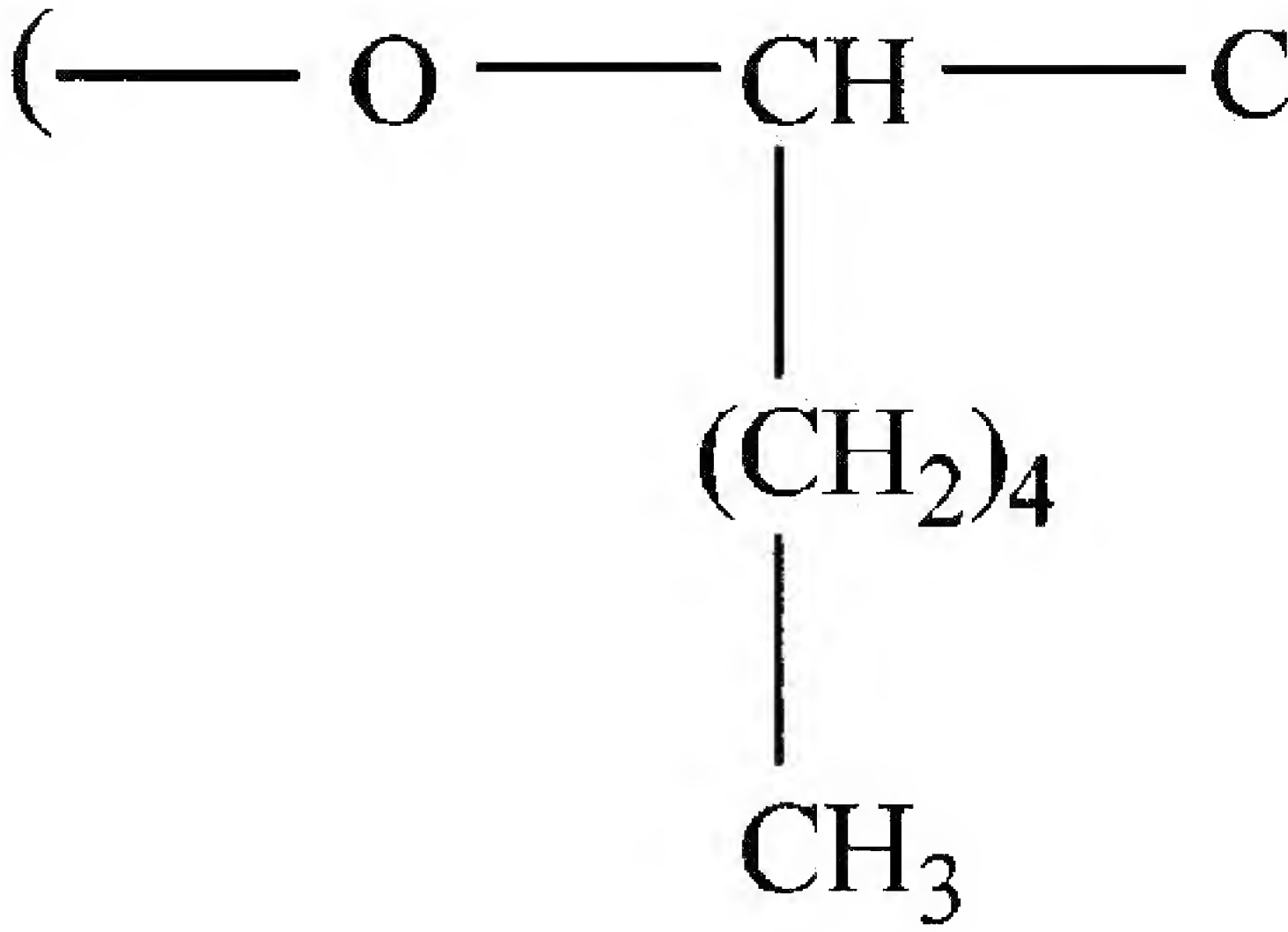
[Chemical formula 3]



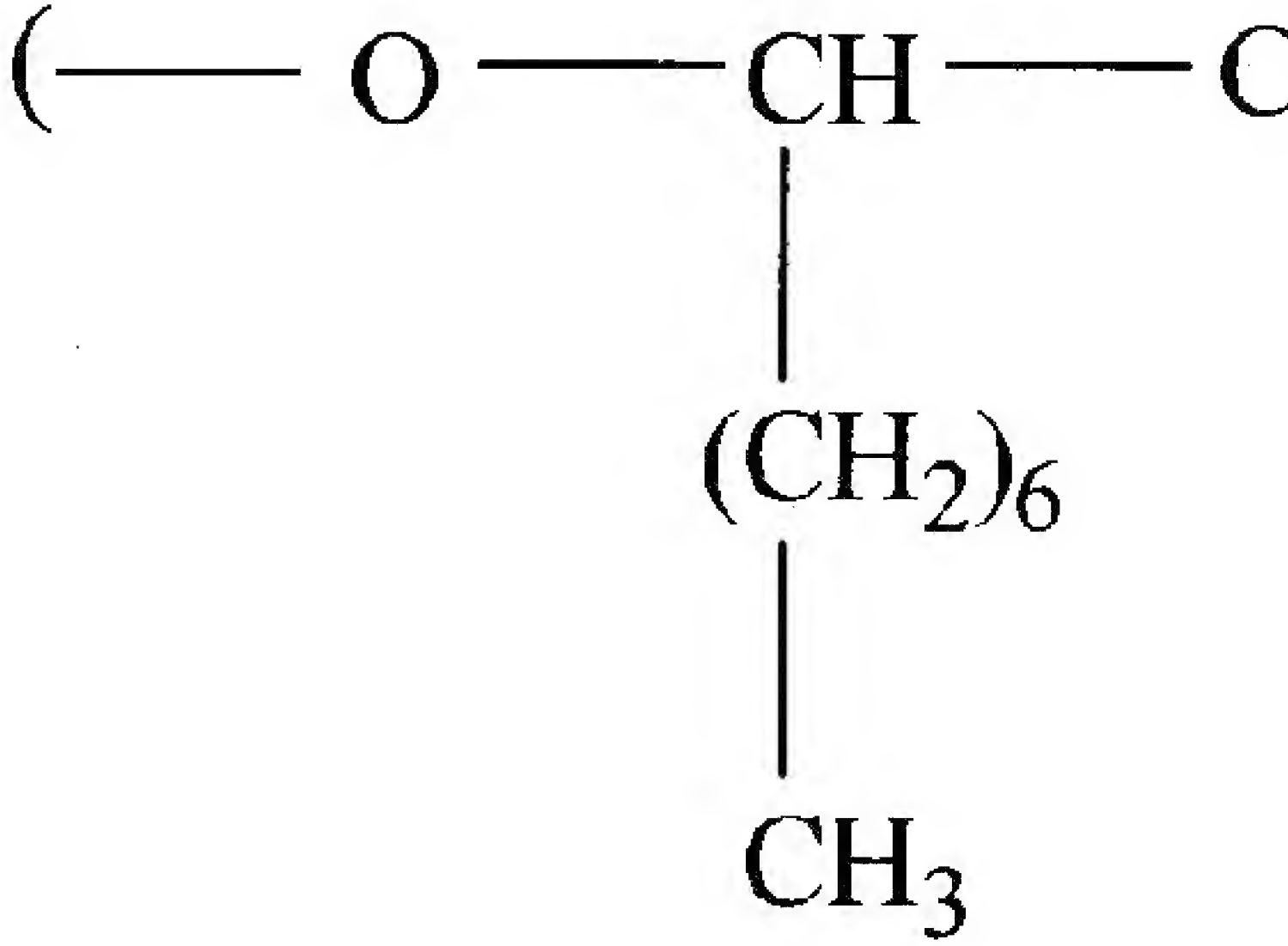
A of the chemical formula 1 0~90% , and the b of the chemical formula 2 are 0~90%. The c of the chemical formula 3 is 10~95%.

Moreover, it is preferable that the above-described monomer is 3- hydroxyoctanoate of the estival chemical formula 4, 3- hydroxy dodecanoate of 3- hydroxy decanoate of the chemical formula 5 and chemical formula 6.

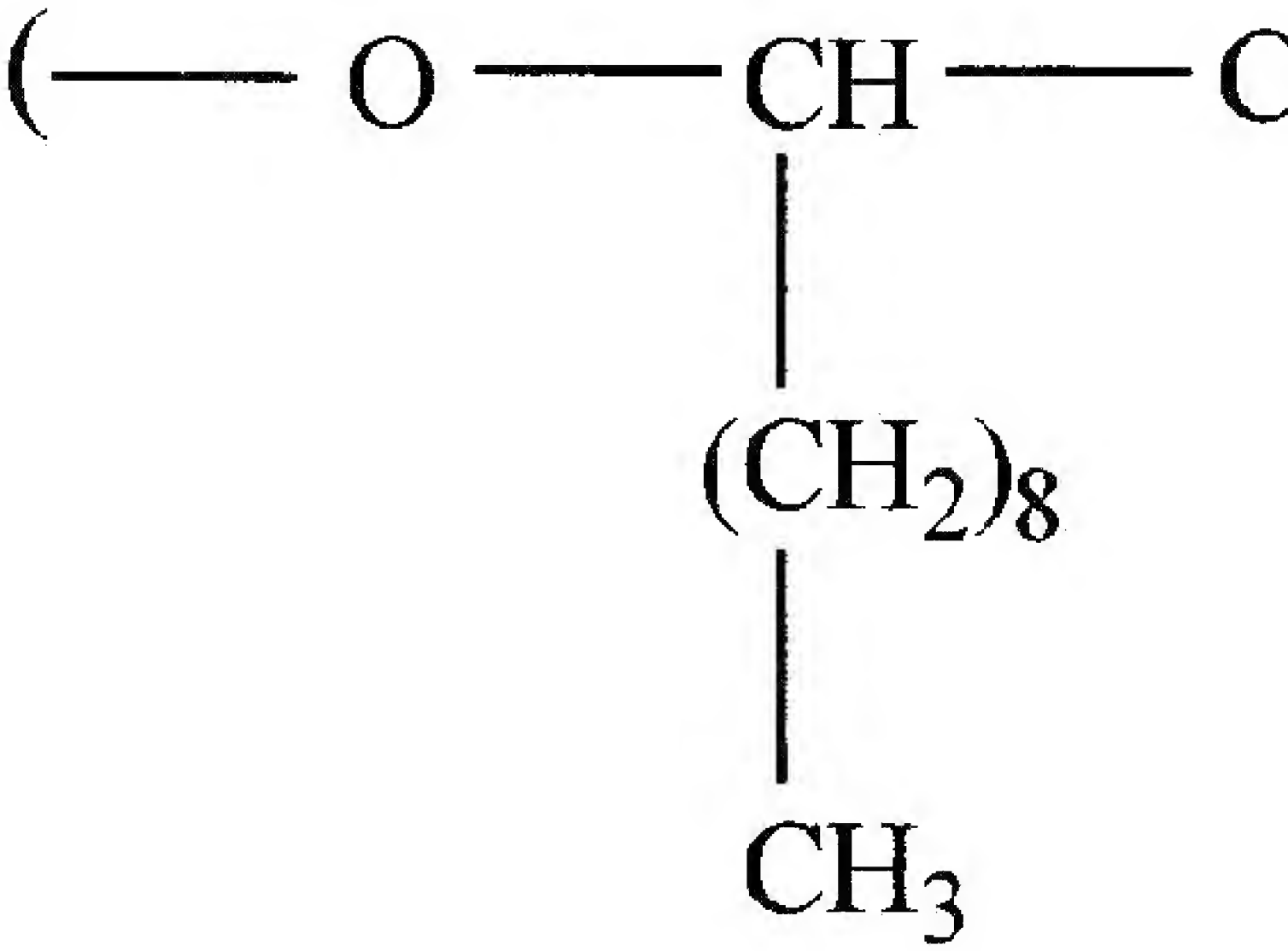
[Chemical formula 4]



[Chemical formula 5]



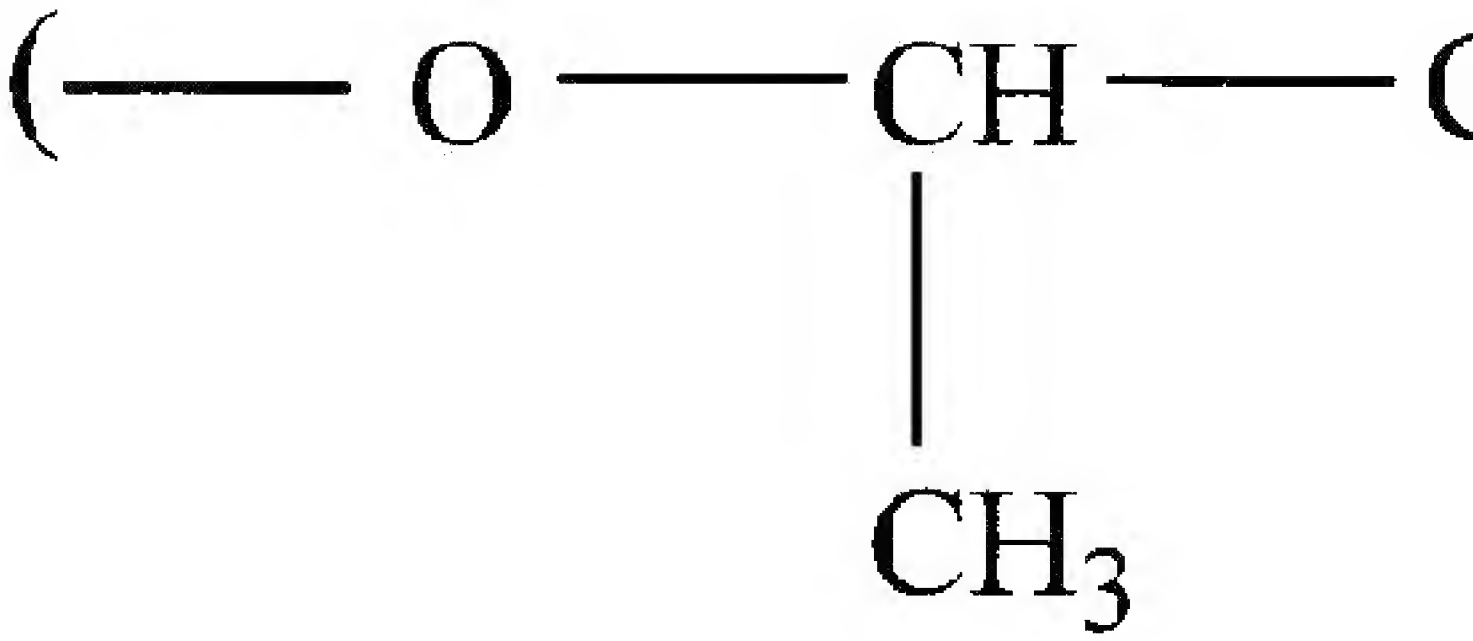
[Chemical formula 6]



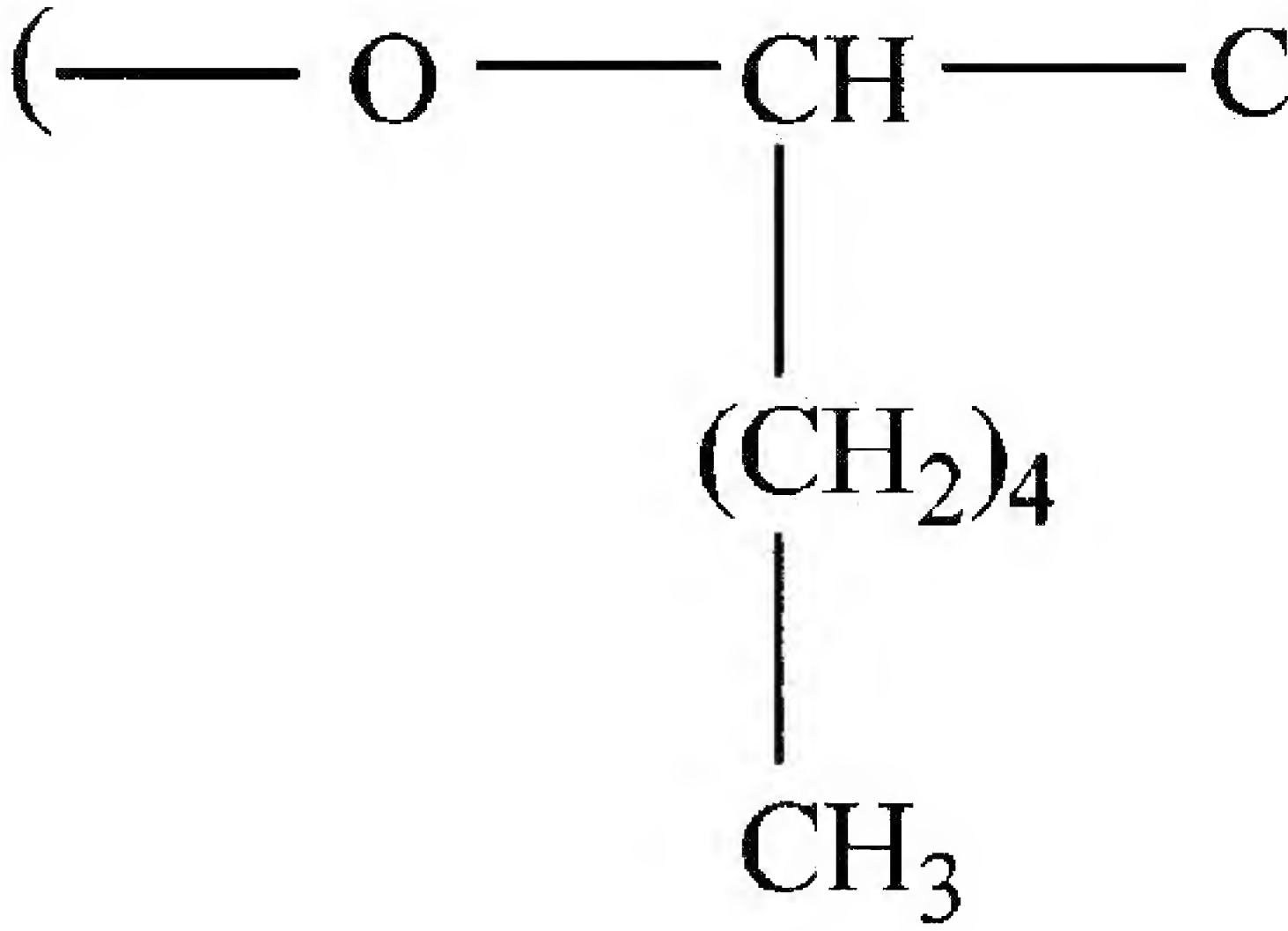
The d of the chemical formula 4 described in the above 0~55% , and the e of the chemical formula 5 are 45~90%. The f of the chemical formula 6 is 0~55%.

Moreover, it is preferable that it is 3- hydroxybutyrate of the estival chemical formula 1, 3- hydroxyoctanoate of the chemical formula 4, 3- hydroxy dodecanoate of 3- hydroxy decanoate of the chemical formula 5 and chemical formula 6.

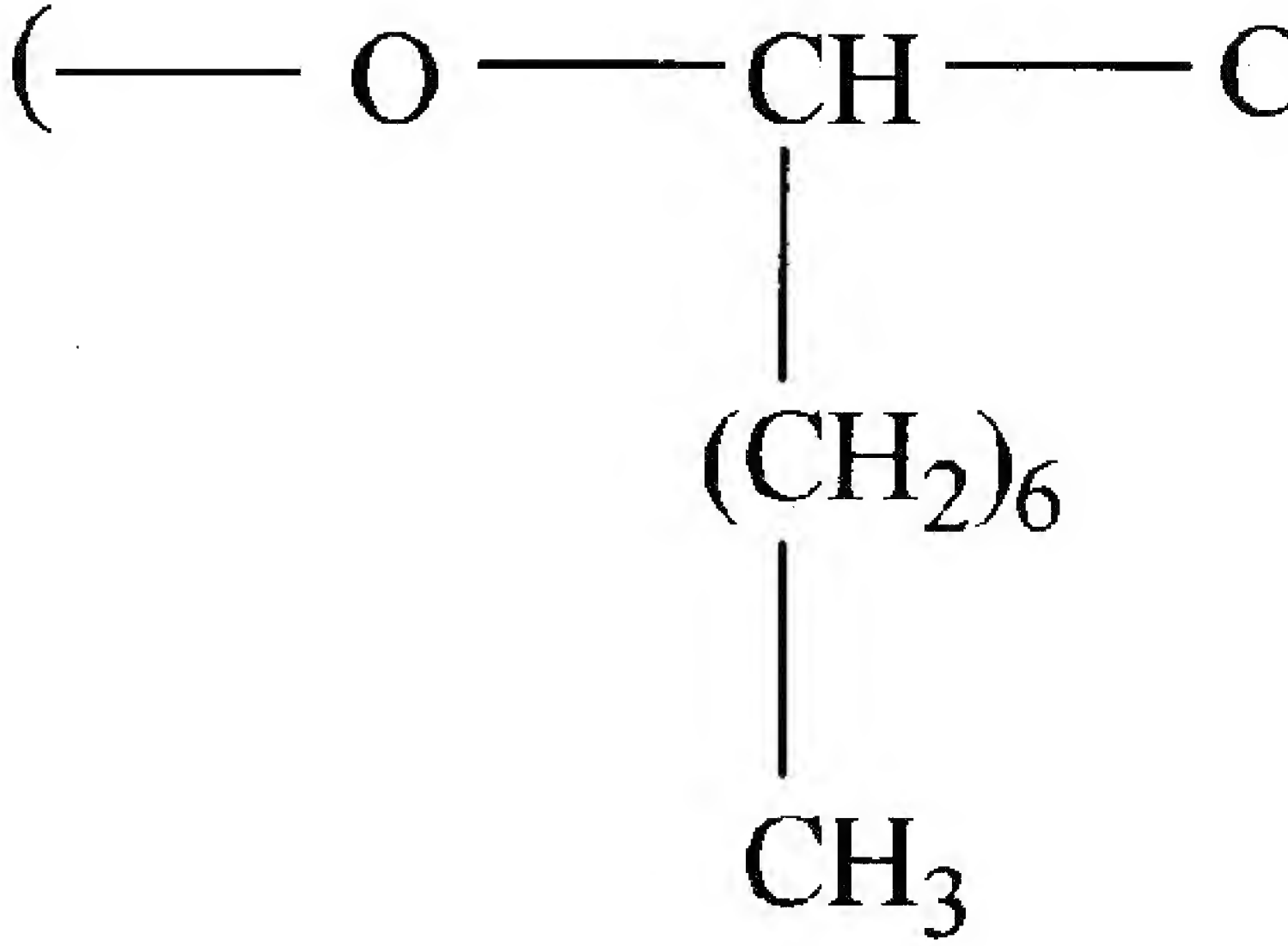
[Chemical formula 1]



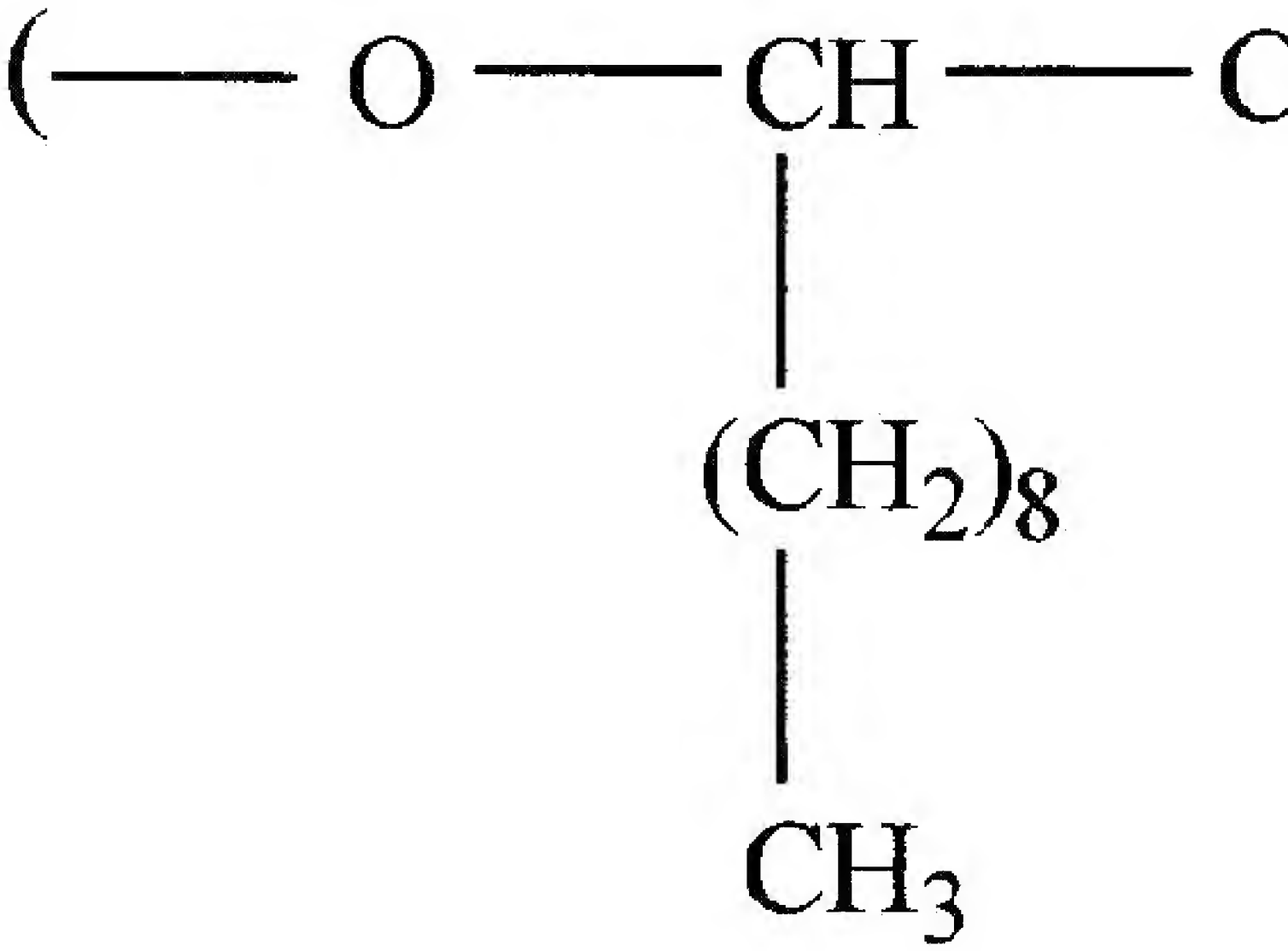
[Chemical formula 4]



[Chemical formula 5]



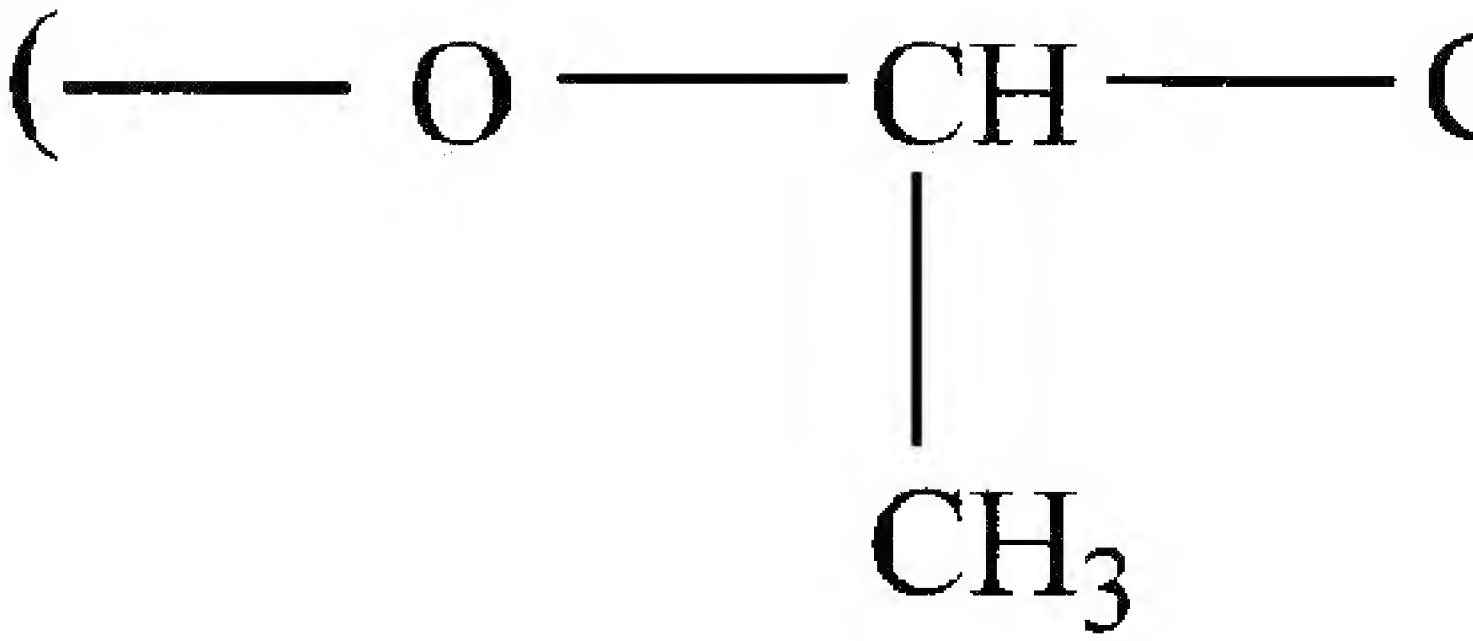
[Chemical formula 6]



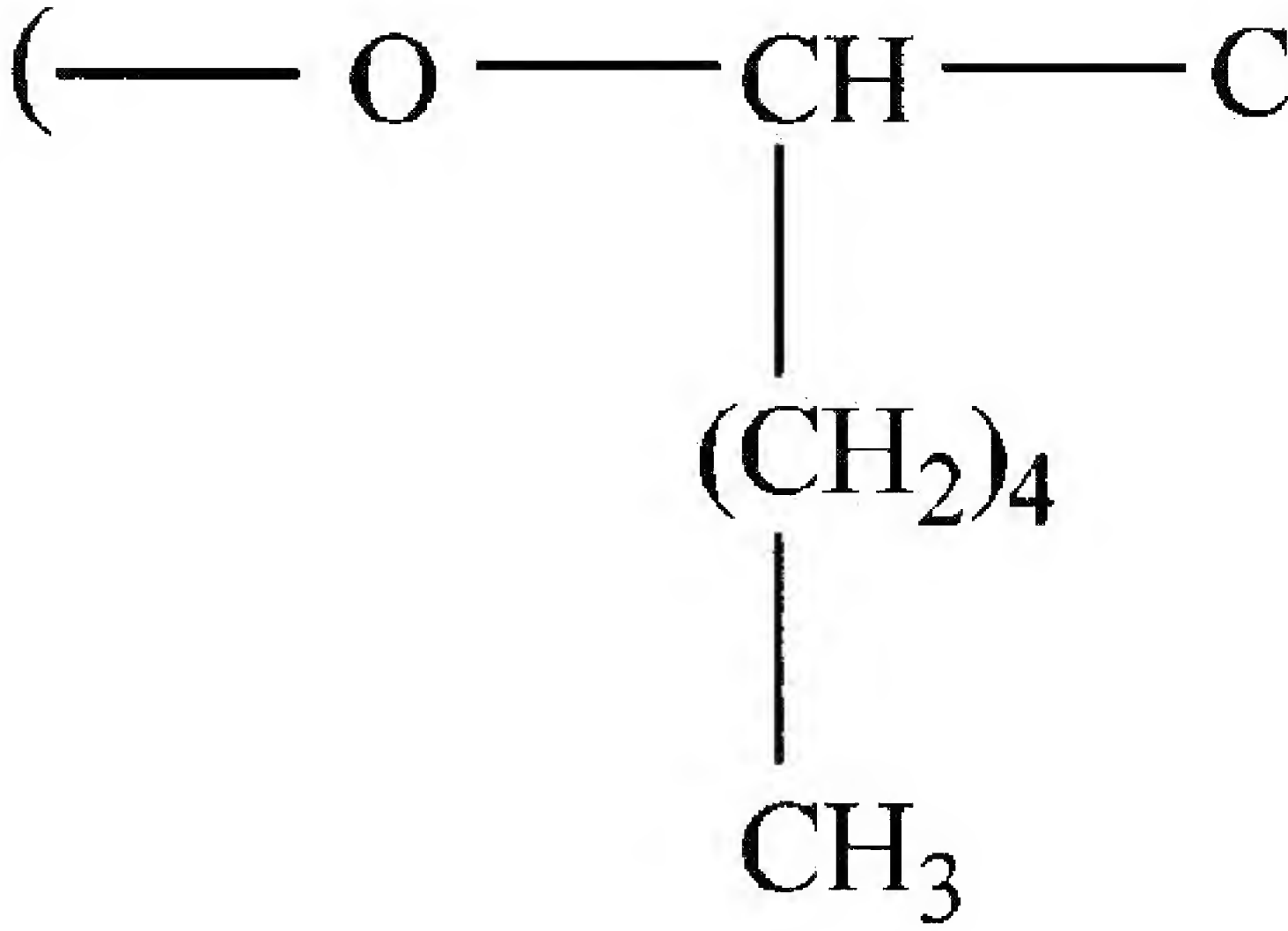
A of the chemical formula 1 described in the above 5~85% , and the d of the chemical formula 4 5~85% , and the e of the chemical formula 5 are 5~85%. The f of the chemical formula 6 is 5~85%.

Moreover, it is preferable that the above-described monomer is 3- hydroxybutyrate of the estival chemical formula 1, 3- hydroxy dodecanoate of 3- hydroxyoctanoate of the chemical formula 4 and chemical formula 6.

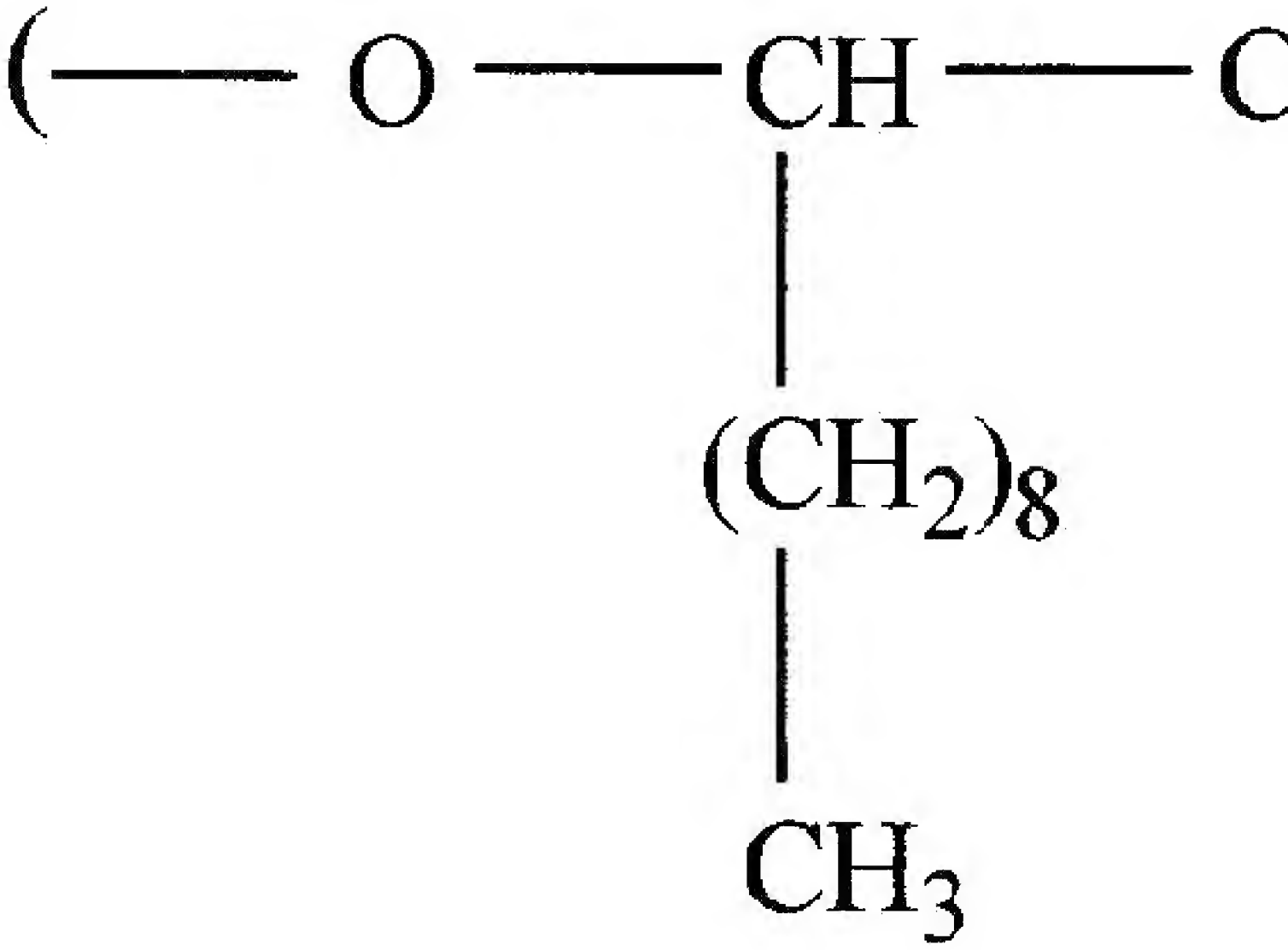
[Chemical formula 1]



[Chemical formula 4]



[Chemical formula 6]



A of the chemical formula 1 described in the above 5~90% , and the d of the chemical formula 4 the f of 5~90% and chemical formula 6 is 5~90%.

As to the invention, and, the other form, the manufacturing method of polyhydroxyalkanoate including the process of cultivating and producing polyhydroxyalkanoate is provided to the culture medium including the unsaturated carboxylic acid and/or the saturation the above-described Pseudomonas sp. HJ-2 strain.

It is preferable that the above-described unsaturated carboxylic acid and/or the saturation is selected in the valerate, heptanoate, the vegetable oil, the animal fat and the group consisting of the fish gasoline than.

Moreover, as to the invention, and, the other form, the elastic body including the above-described polyhydroxyalkanoate copolymer to the main component is provided.

As the above-described elastic body is for medical treatment rubber elastic body, it can use in the medical glove, the rubber band, the packing material or the contraception appliance etc.

And in the present invention, the manufacturing method of the polyhydroxyalkanoate copolymer having the improved pull value including the process of stretching the film which it manufactures by using the above-described polyhydroxyalkanoate copolymer to one side direction or bidirectional in the crystallization former or after is provided.

Moreover, in the present invention, the moldings which is the fiber including the above-described polyhydroxyalkanoate copolymer, and fabric or film-type is provided.

In the invention, the strain using the Pseudomonas sp. HJ-2 separating strains which can grow up by using *** acid (dialkanoic acid) in the sludge and shows the unique with the middle PHAs biosynthesis mode weighs the PHAs from the various carbon source including *** acid (monoalkanioic acid), *** acid etc. with the biosynthesis heartburnings.

When the elastic body of the present invention is manufactured, the property is changed according to composition and processing method of polymer. In this way, the method for controlling the elasticity of the elastic body, it does, different methods can be applied.

When the first method cultivates the Pseudomonas sp. HJ-2 as the same method as the polymer composition way of regulation, the concentration of the carbon source, and the concentration of the concentration of the oxygen or the nitrogen are controlled and since repeater and heavy chain road of the short-chain length PHA control the relatively amount of the repeater of PHA the elasticity manufactures the different material.

There can be the method for processing it crystallizes and two methods for processing after crystallization is run out of as the method which the second method processes the obtained polymer. After it cuts in one in the shape desiring the macromolecular film, after it crystallizes 4~5 hours and it cuts with square, it extends to the extent of the maximum which it does not cut with 200~400% of the original length by using the instron. And one manufactures the material which stretches to the shape desiring the desired film to the extent of the maximum which does not cut with 200~400% by using instron in the amputated after, and the state that is not crystallized and crystallization occurs within the moisture and has the different elasticity.

The third method manufactures the material having the different elasticity which the key face in photocrosslinking is the material which mixes the sensitizer like the benzophenone of polymer and number % or the benzophenone derivative and which it does like the secondly described in the above or the third method and prepared cross-linked in the low temperature using the ice or the dry ice.

Moreover, the film using the invention can manufacture like next. It is polymer the rust in the chloroform, the acetone, ethylacetate etc. This is poured into container including the glass, controlling horizontal metal or Teflon etc. and solvent is gradually let fly and film is manufactured with first.

After the polymer mass is put into the spacer of the desired thickness which is puts between the steel plate coated with two steel plates, for example, Teflon etc. and it heats with the room temperature ~150℃, it presses to the pressure of 10~50 tone and film is manufactured with second.

Before being crystallized, the film manufactured with the first described in the above or the second method is extended and film is manufactured with third. At this time, the pull value is the just before to be destroyed with 100~.

After the film manufactured with the first described in the above or the second manufacturing process of film is crystallized, it stretches to one side direction or bidirectional and film is manufactured with fourth. At this time, the pull value is the just before to be destroyed with 100~.

The sensitizer like the benzophenone of polymer and number % or the benzophenone derivative are mixed and film is manufactured with the above-described method and the ultraviolet ray is irradiated with fifth.

Moreover, it extends the obtained film and it does it tears to the built-in direction in the form of the thread and the fiber using the invention manufactures.

[Embodiment]

Hereinafter, the preferred embodiment of the present invention and comparative example are described. But it is the embodiment of the present invention for helping the understanding of the present invention but the embodiment which it is described below is not restricted to the embodiment which the invention is described below.

Hereinafter, specifically it is the invention the same based on the embodiment like next.

Embodiment.

Separation and identification of the strain Pseudomonas sp. HJ-2.

The Pseudomonas sp. HJ-2 has gram-positive, and oxidase and catalase - positivity lice, the spore non-formation dishonest merchant (rod-shaped) form. In the agar medium, the translucency (transluscent) colony was produced. And the fluorescent dye did not produce on the king B culture medium. And the glaucousness non-fluorescence diffusible pigment was produced on the PCA (or, NA). Moreover, one or more polar flagellums (flagella) was produced. And it grew in 42℃.

And it is the same as that of the following table 1 if the property of the other pseudomonas sp. HJ-2 is organized.

Condition. Result.
The myxopoiesis in sucrose. -
β - galactosidase. -
Arginine dihydrolase. +
Lysine di carboxylase. -
Ornithine decarboxylase. -
Citrate usage knuckle. +
H2S production. -
Urease production. -
Tryptophan deaminase. -
Indole production. -
VP reaction. -
Gelatin liquefaction. -
Oxidase. +
The acid of the glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, arabinose, amygdalin. -
Denitriding. +
β - glucosidase. -
The usage knuckle of α -D- glucose, D- gluconate, caprate, adipate, malate, phenyl acetate. +

Moreover, the strain looked at grew in the citrate, suceinate, twin 40, tween 80, L-arabinose, D- fountain-pen pocket exposuremeter, acetate, cis- aconitate (cis-aconitate), β - hydroxybutylate, P- hydroxy phenyl acetate, itaconate, α - ketoglutarate, quinate, propionate, D,L- lactate, L-alanine, L-asparagin, L- asparate, L-glutamate, hydroxy L-proline, L-proline, L-serine, putrescine, D,L- carnitine, γ - ***, U RoKa NATE (urocanate), inosine, 2- aminoethanol.

The fatty acid profile of the Pseudomonas sp. HJ-2 is same as those of the following table 2.

C18:1 w7c/w9t/w12t 33.2%
C16:0 24.6%
C16: 1 w7c/ C15: 0 iso 2OH. 21.5%
C12:0 2OH 7.4%
C12:0 3OH 3.9%
C10:0 3OH 3.1%
C12:0 3.0%
C17: 0 cyclo. 1.6%
C14:0 1.0%
C18:0 0.4%
C19: 0 cyclo w8c. 0.4%

It looked at with the result as described above and the Pseudomonas sp. HJ-2 consisted of the Pseudomonas caryophylli belonging to the RNA group II with identification.

In the present preferred embodiment, ½ E which the Pseudomonas sp. HJ-2 used obtained from the sludge from the enrichment culture, and the nonanoate for the maintenance of stocks to the carbon source*In the medium plate (media plate), it subcultured 1 week. It deposited the Pseudomonas sp. HJ-2 in Korea Institute of Science and Technology gene engineering center Center for Biotechnology Information Genbank 1997 year November 14 to the deposit number KCTC 0406 BP. ½ E*The composition of the culture medium showed in the following table 1.

In the Pseudomonas sp. HJ-2 is the thermostatic chamber of 30℃, while cultivating in the flask of 2ℓ with 1ℓ by using the hugeness mixer (giant shaker), it put 3ℓ fermentation medium in the container (jar) of 5ℓ and the fermentation performed. Flask and the culture medium which is used when fermenting and culturing is ½ E.*It was the culture medium. It added the same carbon source as the kind of each carbon source used and 10% of each volume became the seed medium which it respected to do the flask cultivation and fermentation. The fermentation performed in the condition of the PH 7.0±0.1, temperature 30.0±1℃, the air flux (air flow rate) 1.0vvm, 300rpm.

½ E*The composition of the culture medium.

Medium component.	Flask medium (g/ℓ)	Fermentation medium (g/ℓ)
NaNH4HPO44H2O	1.75	1.75
K2HPO43H2O	3.75	3.75
KH2PO4	1.85	1.85
0.1M MgSO4·7H2O	10	10
Mineral solution.	5	5
Carbon source.	5	5

In the above case, composition of the mineral solution is same as those of the following table 4.

The composition of the mineral solution.

Component.	Concentration (g/1M HCl 1ℓ)
FeSO4·7H2O	2.78
MnCl2·4H2O	1.98
CoSO4·7H2O	2.81
CaCl2·2H2O	1.67
CuCl2·2H2O	0.17
ZnSO4·7H2O	0.29

Bio resources method of measurement.

By utilizing the monochromator (spectrophotometer, Milton Roy spectronic 120011, U.S.A.), the undiluted solution was diluted with 1/5 and the bio resources was measured in the OD 666㎚.

The cell hobbyist (cell harvest) and freeze-drying.

In the cell obtained to the flask cultivation and fermentation performance is 4℃, while centrifuging for 10 minutes at 10,000rpm and obtaining, by using the freezing dryer (freeze dryer) after freezing in freezer, the obtained cell obtained the dry cell.

The PHAs extraction and refinement.

After by the dry cell and the sand on a beach (sea sand) frozen and dries being together put in the mortar and going, putting into the timble filter (thimble filter) and using the chloroform, the PHAs was extracted in 12 for hour Soxhlet extrator (soxhlet extractor). By using methanol, the PHAs extracted was made laboriously. The PHAs repeating the process with two or three burn and was refined was obtained.

The composition analysis of the PHAs.

The PHAs 5㎎ and the chloroform 1㎖ refined in the Pyrex cap tube for GC, and 15% H.2SOAfter the methanol 1㎖ in which 4 was contained being put and doing with the vortexing (vortexing), it played in 105℃ drying oven with 2 for hour methanolysis (methanolysis). After completely cooling after taking out of the drying oven, the distilled water 1㎖ was added and it vortexed strongly and the chloroform layer was used for the analysis. The condition of the PHAs gas chromatography was organized in the table 5.

Item. Content.
Model system (model system) Hewlett Packard 5890 series II
Detector (detector) Flame Ionization Detector(FID)
Column (column) Capillary HP-1, 25mID 0.2㎜ (narrow bore)
Liquid phase (liquid phase) 100% dimethyl polysiloxane.(demethyl polysiloxane, Gum)
Solid support (solid support) Chromosorb PAW DMCS
Input and detection port temperature.(Inj. & Det. port temp.) 240/270℃
Carrier gas (carrier gas) N2
Air / hydrogen flow velocity (air/hydrogen flow rate) 350/35㎖/min
Aggregate rate (total flow) 102㎖/min
Septum purge vent speed.(septum purge vent flow) 1㎖/min
Column head pressure (column head pressure) 1㎖/min
The part is the gas speed.(auxiliary(make-up) gas flow) 29㎖/min
The initial temperature and time. 80℃/4min
Temperature ramp-up rate (temp. up-grade rate) 10℃/min
The final temperature and time. 230℃/3min
Solvent amount of administration (solvent & inj. size) CHCl3(Chloroform) /1㎕.
Input port mode (injection port mode) Division mode (split mode)
Division ratios (split ratio) 100 to 1

Embodiment 1: the PHA synthesis from the sugar.

100% PHB homopolymer was biosynthesized in the result glucose decreasing the glucose and gluconate to the respective single carbon source and cultivated the Pseudomonas sp. HJ-2 in 2ℓ flask with 1ℓ. And for the heavy chain road, the PHAs (3HO, 3HD, 3HDD) was biosynthesized in gluconate with PHB. The PHA summation result is the same as that of the following table 6 with the Pseudomonas sp. HJ-2 from the sugar.

The synthesis of PHA from the sugar by the Pseudomonas sp. HJ-2.
Carbon source (g/ℓ) DCW(g/ℓ) PHAs(wt%) PHA composition.
C4 C5 C6 C7 C8 C9 C10 C11 C12

Glucose (10)	1.21	5.73	100					
Fructose (10)	0.98	17.85			29.23	60.87	9.9	
Gluconate (10)	0.79	11.45	76.73			5.29	15.31	2.47

Embodiment 2: the PHA synthesis from *** acid.

The PHAs consisting of octanoate PHB of the carbon number was mainly made in the even number individual butylate, hexanoate, octanoate, decanoate, and 3HO of the respective 8.5% along with 3HB and 22.3% in decanoate was biosynthesized. When giving *** acid in which the carbon number was the odd number as the single carbon source, 3HV was mainly biosynthesized in the valerate which was 5 carbon numbers and for the heavy chain road of 3HN and 3HHp, the PHAs was biosynthesized in the nonanoate in which the PHAs of the poly (3HB-co-3HV-co-3HHp) form was 9 carbon numbers in the heptanoate which was 7 carbon numbers (table 5). Thus, it looks at and the Pseudomonas sp. HJ-2 can know the biosynthesis box the PHAs of the length corresponding to the carbon number of the carbon source classifying the carbon number of the given carbon source and is the Polymer of PHB given in the even number start the deliberation of the bill introduced carbon number in the carbon number of the odd number and biosynthesis. Particularly, it does not pass the PHAs having through the de novo biosynthesis of fatty acids process of having and the biosynthesis can think the PHAs longer than the length which is regarded as thing and is given of number PHAs having the length in which the biosynthesis summer solstice nots and which it looks and it is short than the length of the carbon source which is given with β - oxidation when giving *** acid as substrate or coming under the length the PHAs having the length more than the carbon number of the carbon source which is given when giving the carbon source having the carbon number of the odd number.

The synthesis of PHA from *** acid by the Pseudomonas sp. HJ-2.

Carbon source (mM/ℓ)	DCW(g/ℓ)	PHAs(wt%)	PHA composition.					
C4	C5	C6	C7	C8	C9	C10	C11	C12
Butylate (56.75)	1.54	11.91	100					
Valerate (48.96)	1.16	36.03	6.3	93.7				
Hexanoate (43.04)	2.11	25.17	100					
Heptanoate (38.40)	0.64	17.03	21.6	43.1	35.3			
Octanoate (34.67)	2.38	22.82	91.5	?1	8.5			
Nonanoate (31.60)	0.53	12.57	?1	?1	22	78		
Decanoate (29.02)	1.12	42.95	80.64		11.2	8.16		

Embodiment 2: the PHA synthesis from *** acid.

The PHB homopolymer was biosynthesized when giving the carbon source having the carbon of the odd number in *** acid as the single carbon source. That is, when the di heptanoate (diheptanoate) and *** (dinonanoate) being decreased to the single carbon source and cultivating in the flask of 2ℓ with 1ℓ, the PHB homopolymer was altogether biosynthesized. In the meantime, it is seen that the heavy chain road having 3HO, and the composition of 3HDD and 3HD it gave as the single carbon source synthesize the PHAs the dioctanoate having the carbon of the even number (table 8).

The synthesis of PHA from *** acid by the Pseudomonas sp. HJ-2.

Carbon source (mM/ℓ)	DCW(g/ℓ)	PHAs(wt%)	PHA composition.					
C4	C5	C6	C7	C8	C9	C10	C11	C12
Di heptanoate.(31.22)	1.26	8.61	100					
Dioctanoate.(28.70)	0.73	14.11				20.79	64.20	15.01
***.(26.56)	0.94	23.46	100					
Di decanoate.(24.72)	0.84	5.55				26.23	61.97	11.8

The result coming from *** acid shows the phenomenon of opposite and the result shown in *** acid. In the di alkanoate having the even number start the deliberation of the bill introduced carbon number the PHB homopolymer is biosynthesized in *** acid in the even number start the deliberation of the bill introduced

carbon source, on the other hands, the PHB homopolymer is biosynthesized in *** acid in the carbon source of the odd number, it was the PHAs having the monomer of the carbon number longer than the given substrate the biosynthesis. With synthesizing the PHAs through the de novo biosynthesis of fatty acids process it can think to make the PHAs having the carbon number longer than the length of the given carbon source (the equation 2). It more has with one but as to difference between *** acid and *** acid, -OH is β - oxidation process regarded in the side chain of *** acid due to the difference in *** acid that the PHAs is biosynthesized in *** acid through the de novo biosynthesis of fatty acids process. With being the substrate specificity because toward the given carbon source of the enzyme which probably probablies the PHAs the biosynthesis but engages this is considered.

Embodiment 3: the fermentation at heptanoate.

Heptanoate was decreased to the single carbon source and the blanket supply fermentation (fed batch fermentation) was performed in 30℃, 300rpm. In the blanket supply fermentation, the carbon source is started with 2.8g / ℓ and while in amount of the altogether used carbon source, 5.2g / ℓ was, the substrate consumption amount can know at fig. 1 at the incubation time 30 time obout to be used up over 80%. In fig. 1, the optical density (Optical Density: OD) at 660㎚ is shown. The remaining substrate is shown. One GC result showed the composition analysis of PHA in fig. 2. Referring to Figure 2, the composition was analyzed into 3HB, 3HV, 3HHp. It sampled 100㎖ to hourly and the PHAs was extracted and the monomer composition was found out (fig. 2). It is seen that it irrespective altogether has the monomer composition ratio of 3HB:3HV:3HHp=50:30:20 with the time sampled in the sample of 4 in the fixed rate. In this, the Pseudomonas sp. HJ-2 heptanoate, when making PHA, one PHA monomer is preferred and firstly it synthesizes and one is not later synthesized but each is informed by the fixed rate at the same time, to the biosynthesis heartburnings sweet. Moreover, there can be the terpolymer PHAs and the difference in which the rate of the monomer which is comprised the terpolymer which is known in preexistence because each monomer occupies the concentration having with significance is low as 3HB:3HV:3HHp=50:30:20. It is seen that the general heavy chain road which is lower than that of PHB which is 180℃ to melt as 110.76 ℃ (the drawing 4) to melt and as a result of irradiating the property PHA which obtains from heptanoate in DSC is 40~60℃ is higher than the PHAs.

While the biosynthesis thing shows the PHB homopolymer when the Pseudomonas sp. HJ-2 gave the glucose, butylate, hexanoate etc. as the single carbon source to be similar to the composition function from the most of short-chain length PHA biosynthesis strains including the A. eutrophus, it shows that for 3HB and heavy chain road, the monomer (3HO, 3HD, 3HDD) is biosynthesized in gluconate to be similar to the PHA composition function of the P. aeruginosa. Moreover, in octanoate, it is similar to the PHA composition function from the general Pseudomonas including the P. oleovorans that 3HN is biosynthesized in nonanoate while 3HO is biosynthesized and 3HHp is biosynthesized in heptanoate. Finally, in heptanoate, the biosynthesis as to thing, the Rhodococcus ruber the biosynthesis is the poly (3HB-co-3HV-co-3HHx) the poly (3HB-co-3HV-co-3HHp) similar from hexanoate to function. Like this, the Pseudomonas sp. HJ-2 evenly has features shown in the different strain. Moreover, as seen in the result of showing in *** acid and the result of showing in *** acid, it has to have the investigation about the PHA- biosynthetic pathway that the germ has. That is, PHAs have to be found out even about how using β - oxidation process and de novo biosynthesis of fatty acids process in the biosynthesis. Various carbon sources are assembled and PHAs consisting of the various monomer are produced and it has the research about the optimum conditions about the property of PHA according to the composition and applicability which respects the specific PHA from the Pseudomonas sp. HJ-2 with the biosynthesis below while conducting researches among progressing.

▩ Effects of the Invention

The bio compatibility, and the biodegradation property and elasticity of as to the PHA, in which the length of the concatenation which it manufactures by using the Pseudomonas sp. HJ-2 has various monomers of compositions are excellent. The concentration can be used than the conventional PHA in the different health care technical field by controlling the elasticity according to the concentration of the carbon source which is temperament. Particularly, by the bio compatibility being excellent and replacing with the non-degradable rubber used in the artificial breast or the rubber and using the problem of non-degradable can be resolved.

Scope of Claims

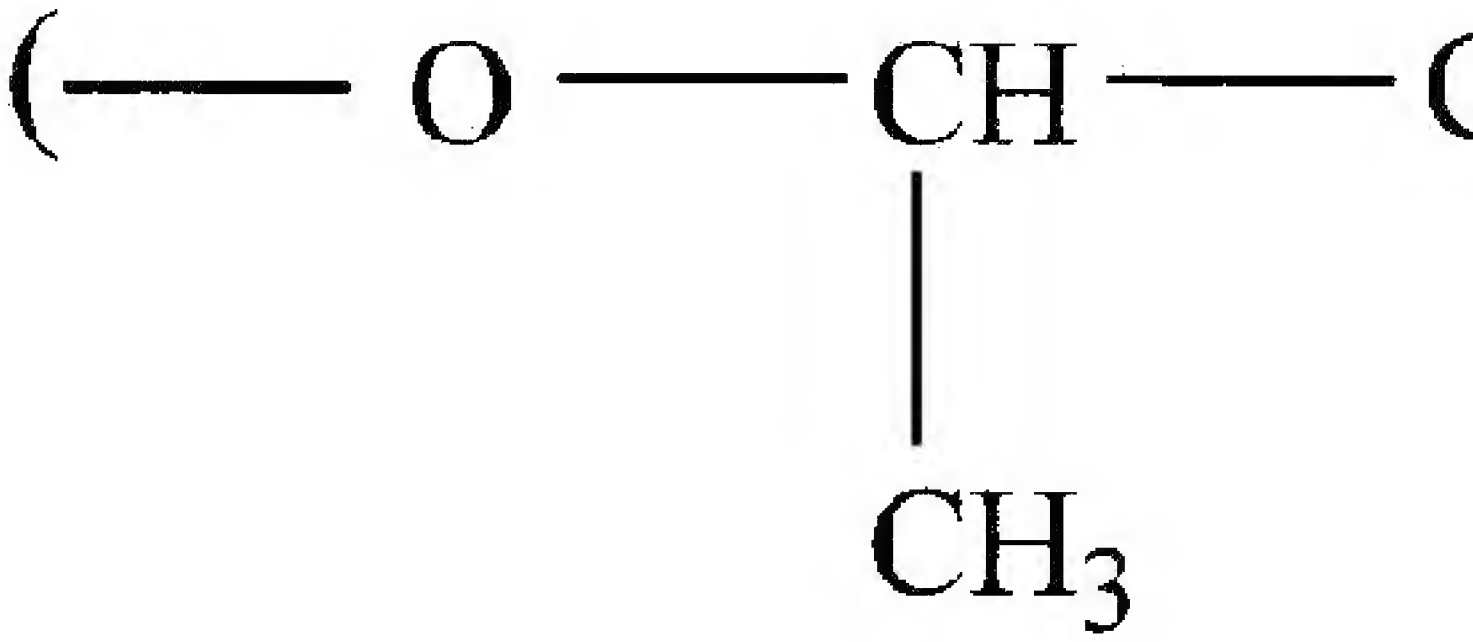
Claim 1 :

C4, C5, C6, C7, C8, C9, C10, C11, C12, C13And c14One selected from the group consisting of monomer or the Pseudomonas sp. HJ-2 strain which is the monomer described in the above deposited in Korea Institute of Science and Technology gene engineering center Center for Biotechnology Information Genbank producing the polyhydroxyalkanoate copolymer included as the main monomer to the KCTC 0406 BP.

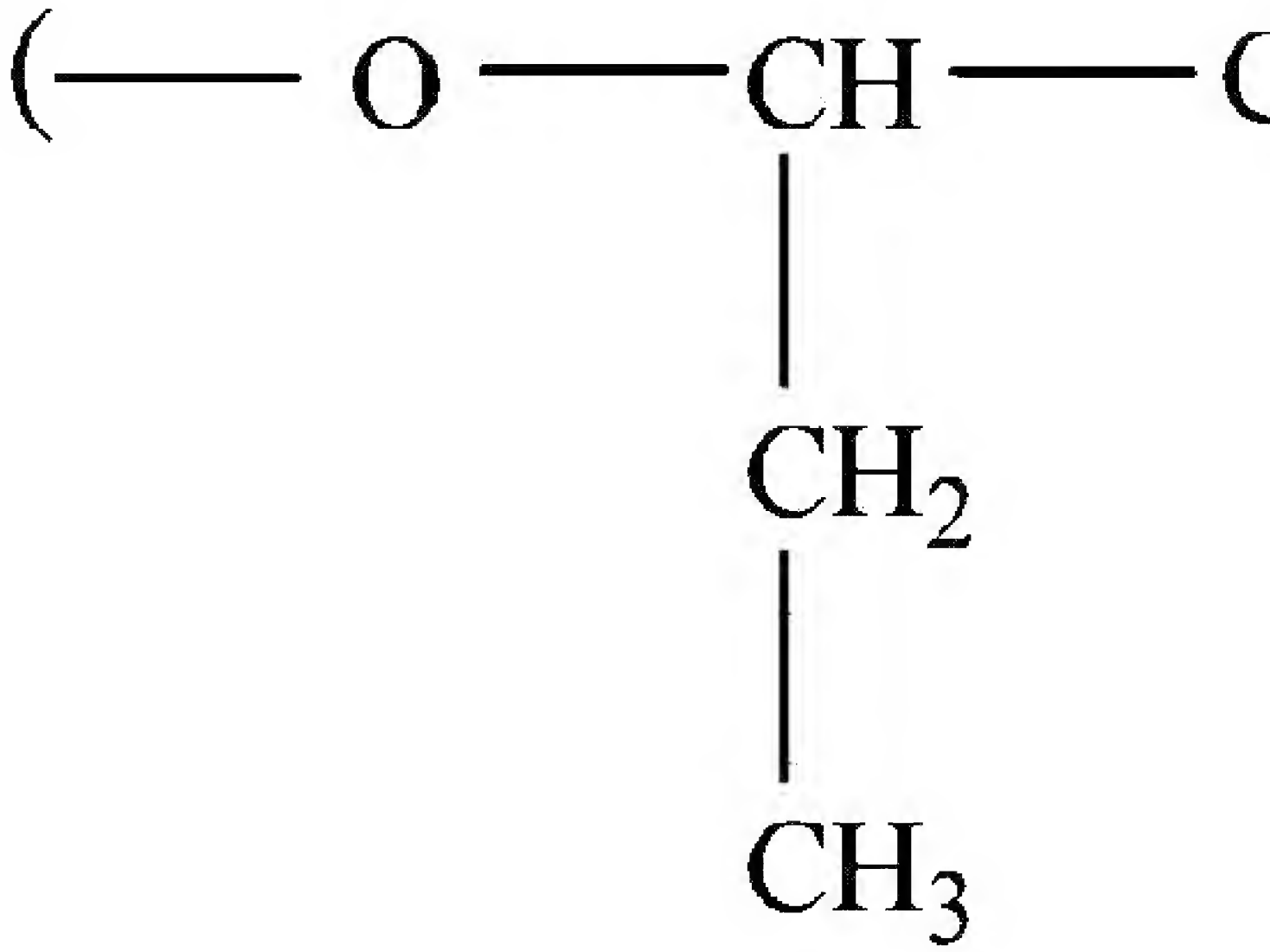
Claim 2 :

The Pseudomonas sp. HJ-2 strain of claim 1, wherein monomer is 3- hydroxybutyrate of the estival chemical formula 1, 3- hydroxy heptanoate of 3- hydroxyvalerate of the chemical formula 2 and chemical formula 3.

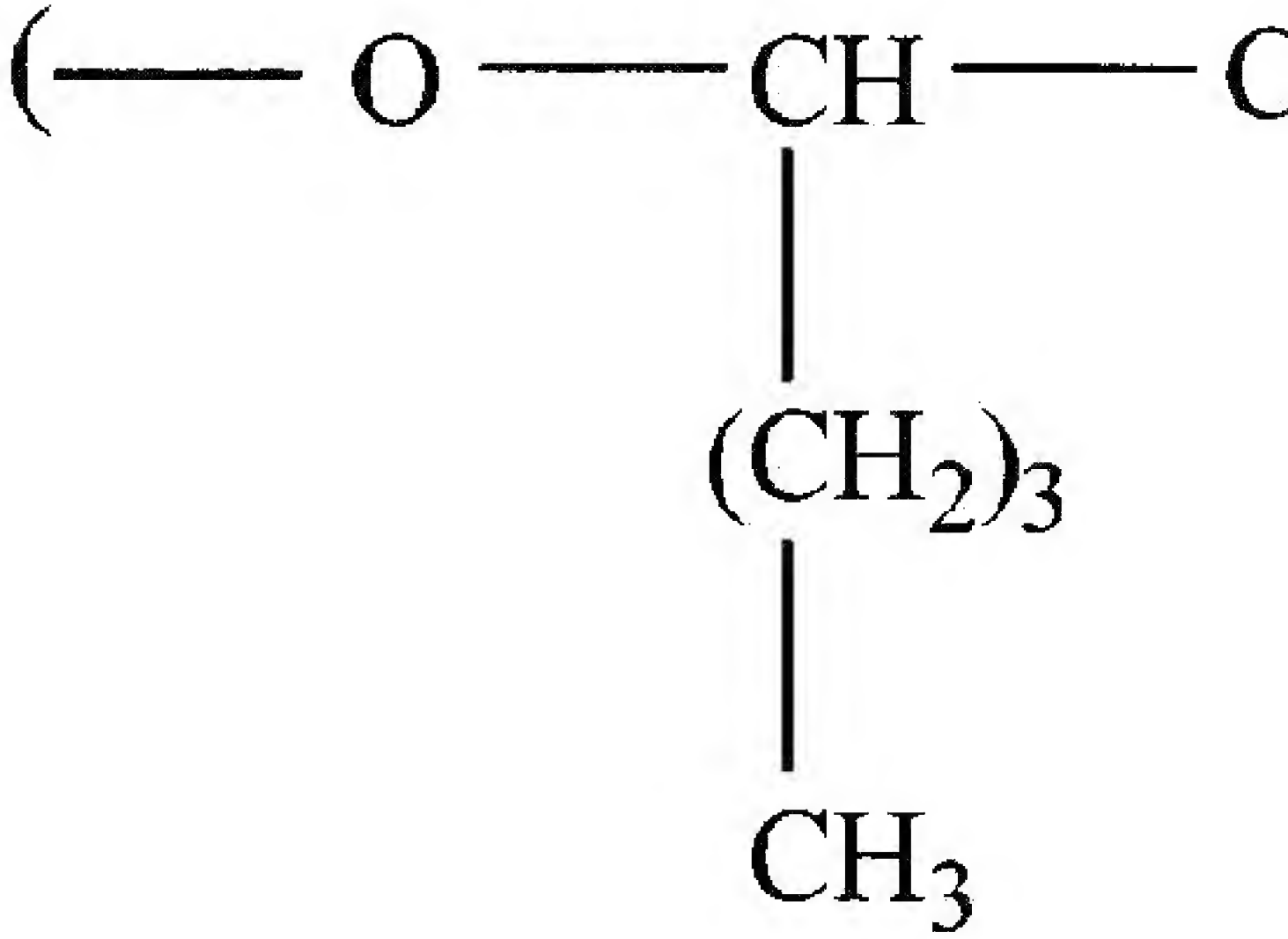
[Chemical formula 1]



[Chemical formula 2]



[Chemical formula 3]

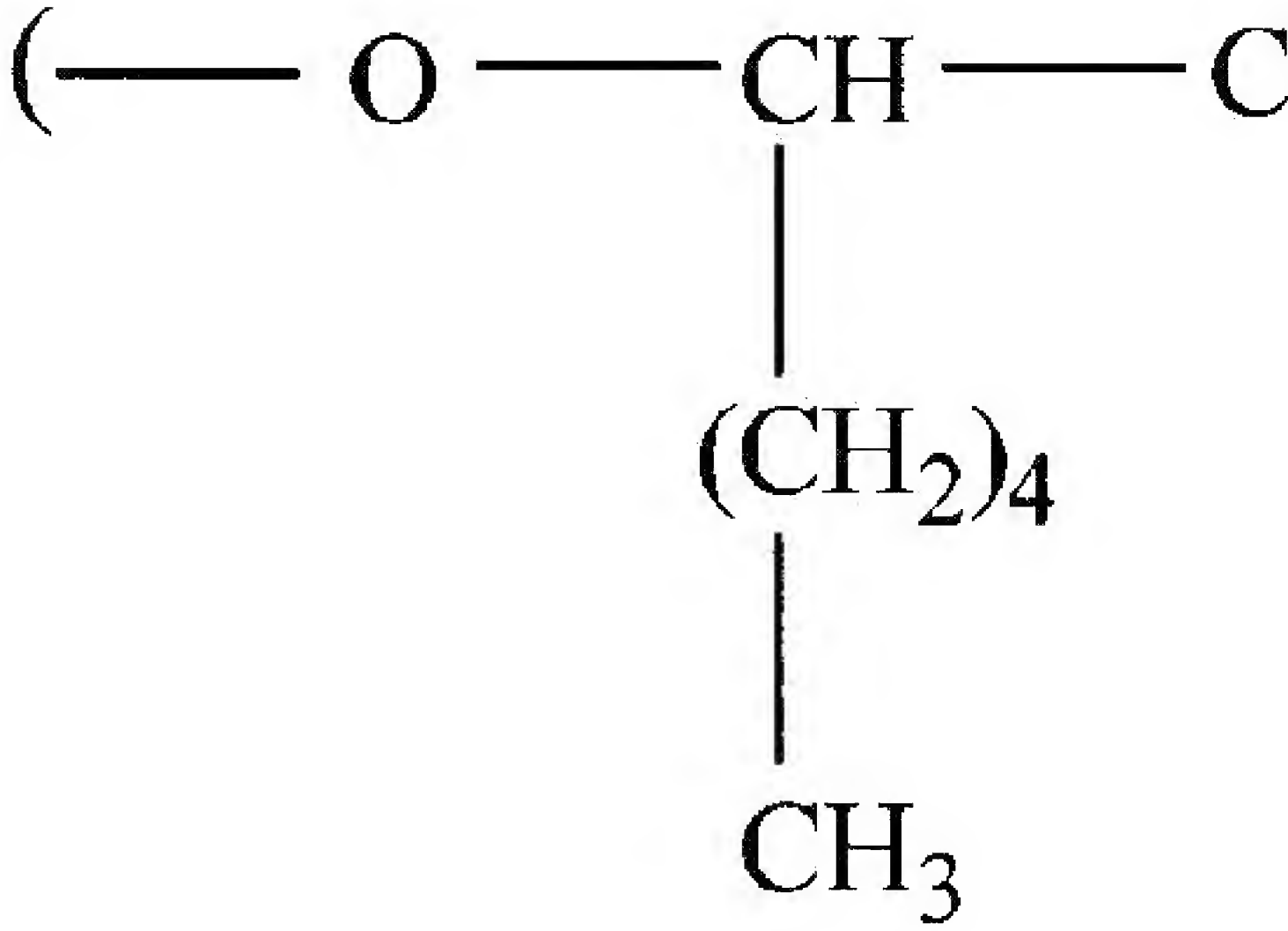


A of the chemical formula 1 5~100% , and the b of the chemical formula 2 are 0~95%. The c of the chemical formula 3 is 0~80%.

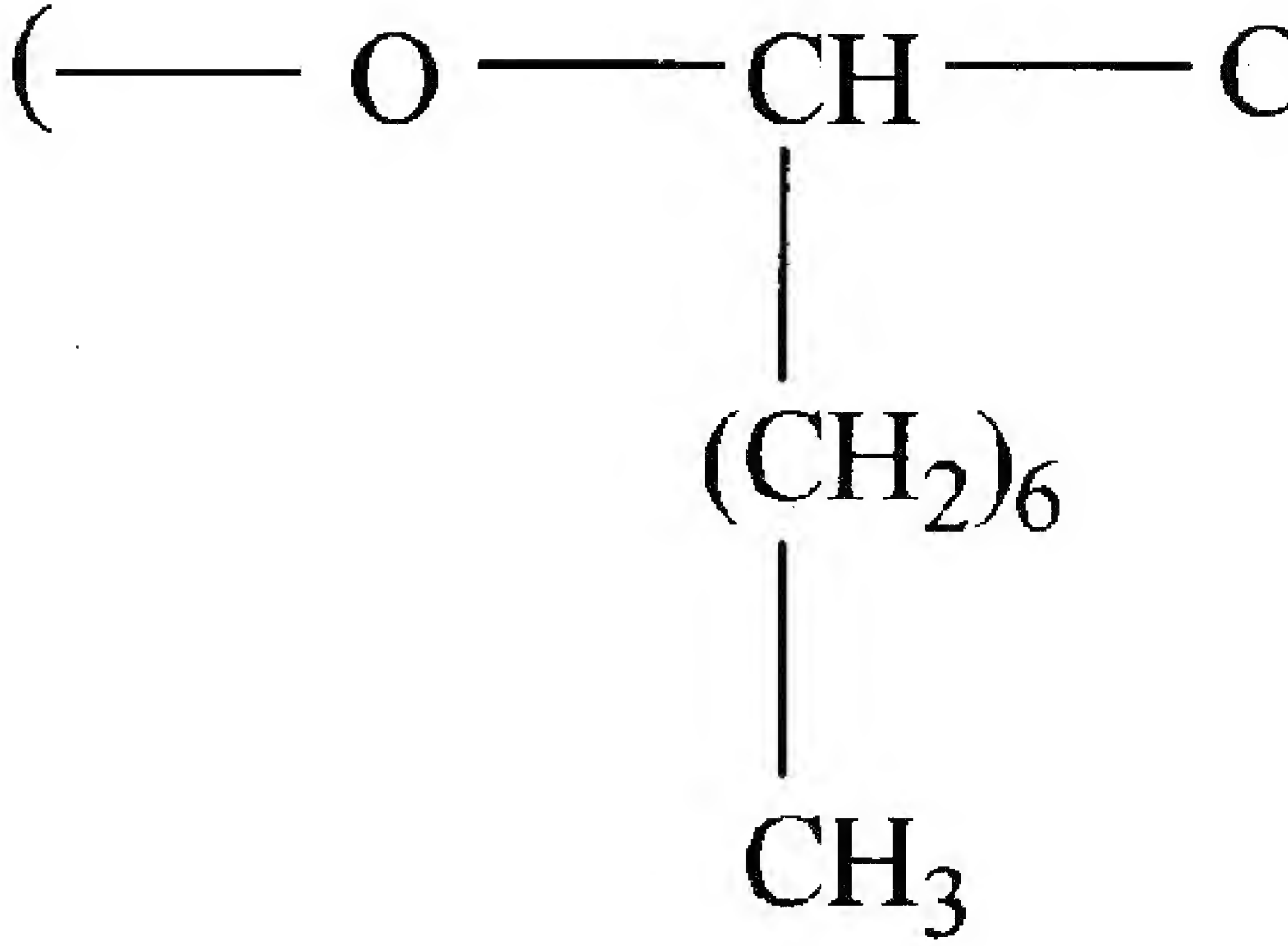
Claim 3 :

The Pseudomonas sp. HJ-2 strain of claim 1 or 2, wherein monomer is 3- hydroxyoctanoate of the estival chemical formula 4, 3- hydroxy dodecanoate of 3- hydroxy decanoate of the chemical formula 5 and chemical formula 6.

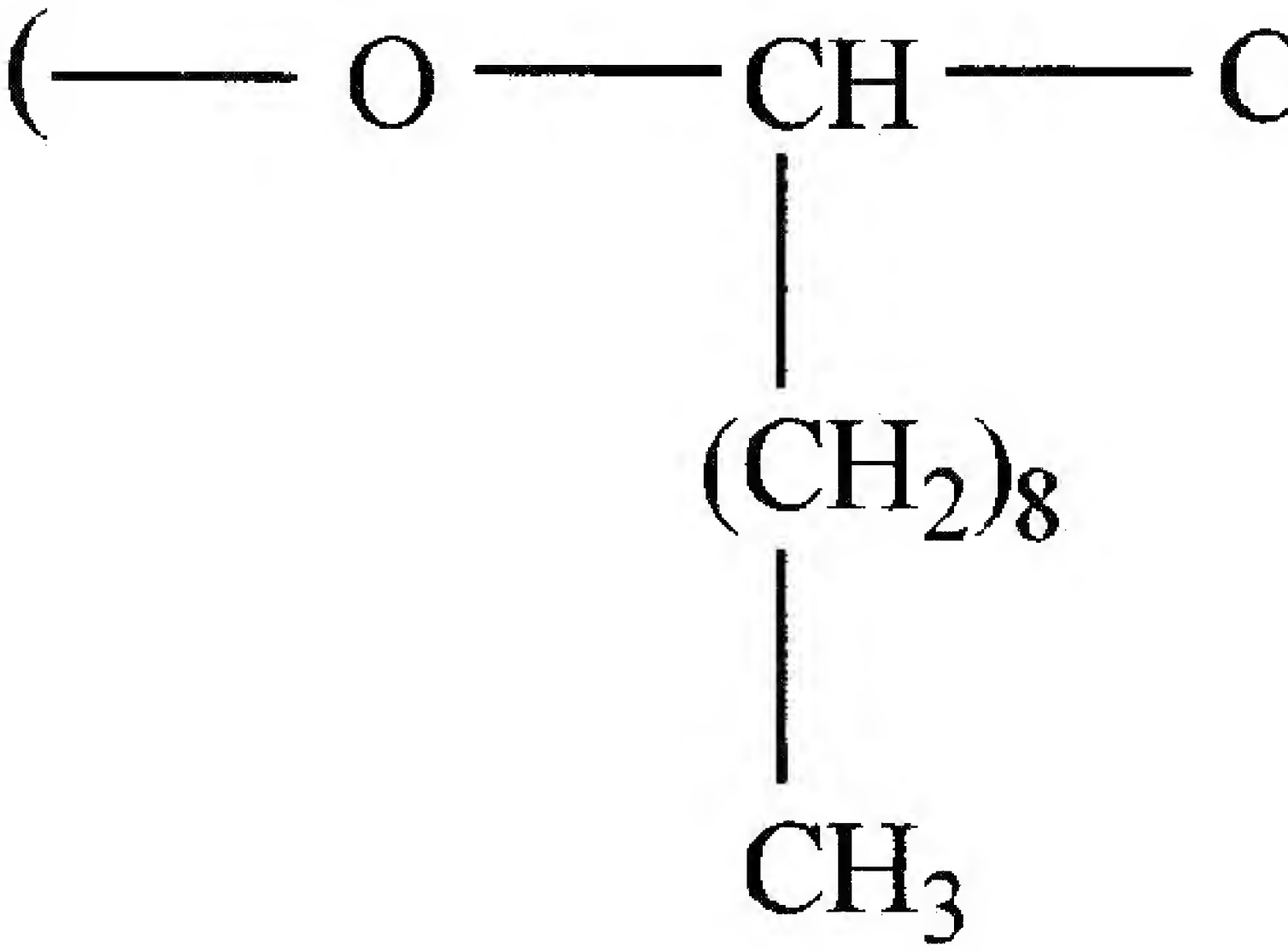
[Chemical formula 4]



[Chemical formula 5]



[Chemical formula 6]



The d of the chemical formula 4 described in the above 0~100% , and the e of the chemical formula 5 are 0~100%. The f of the chemical formula 6 is 0~100%.

Claim 4 :

The Pseudomonas sp. HJ-2 strain in which the culture medium including the unsaturated carboxylic acid hydrolyzing the gasoline selected from the group and is obtained and/or the saturation is to the carbon source and synthesizing polyhydroxyalkanoate of any one of claims 1 through 3, wherein strain is made of the vegetable oil, and the animal fat and fish gasoline.

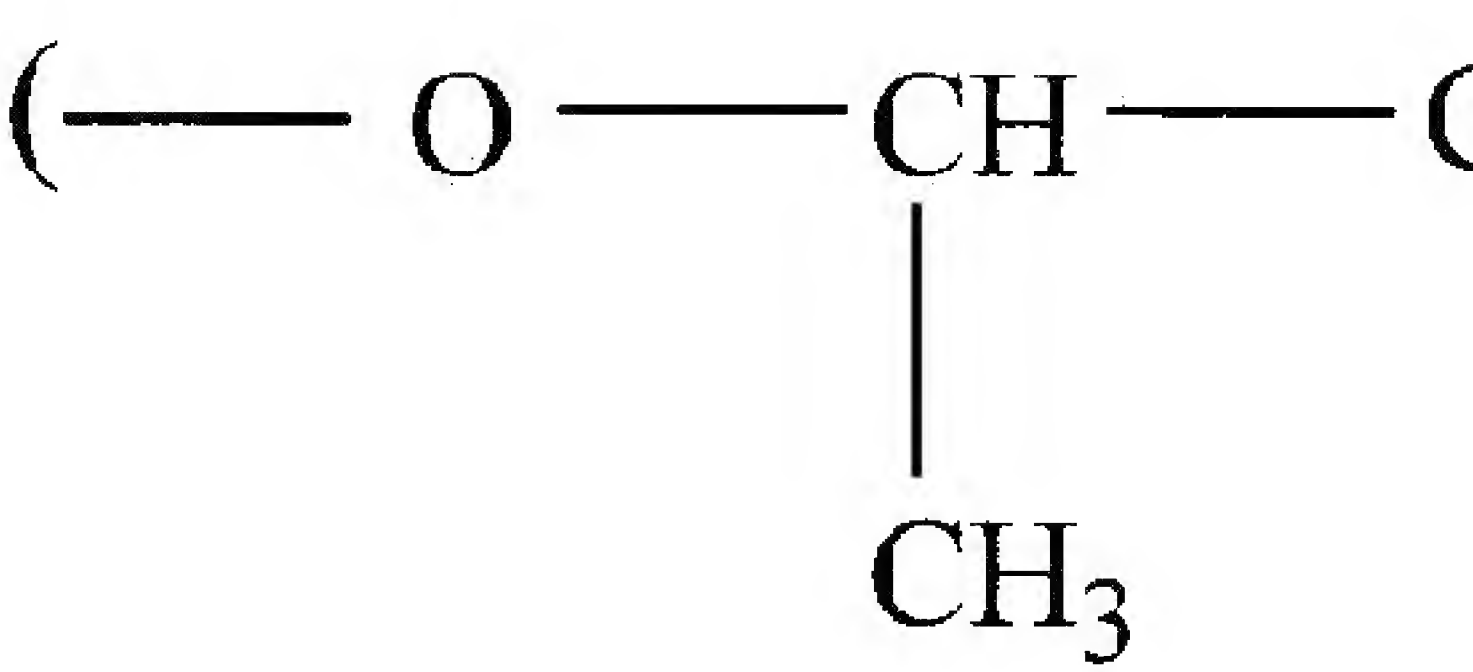
Claim 5 :

C4, C5, C6, C7, C8, C9, C10,C11, C12, C13And c14Two selected from the group consisting of monomer or the polyhydroxyalkanoate copolymer including the monomer described in the above to the main monomer.

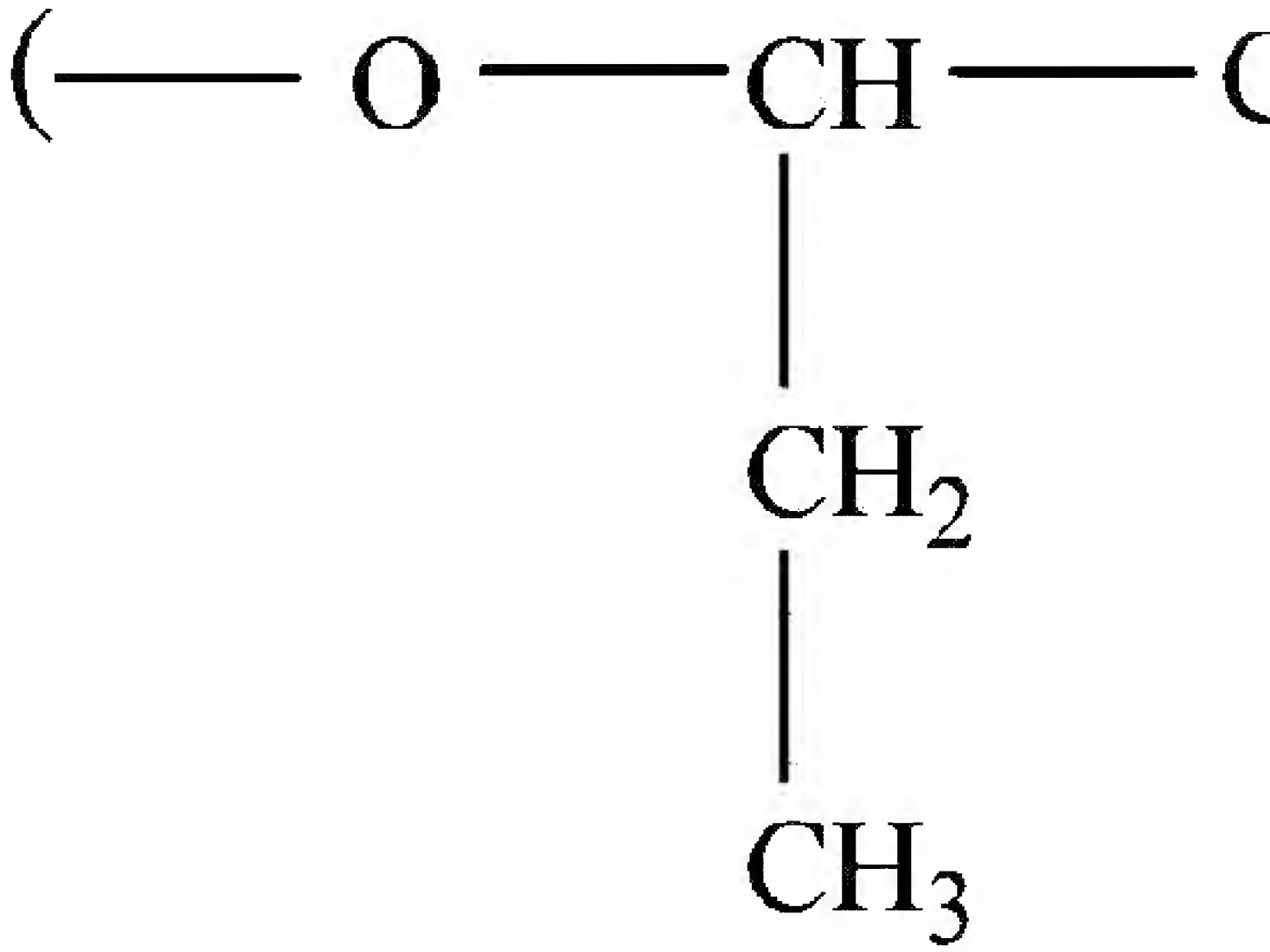
Claim 6 :

The polyhydroxyalkanoate copolymer of claim 5, wherein monomer is 3- hydroxybutyrate of the estival chemical formula 1, 3- hydroxy heptanoate of 3- hydroxyvalerate of the chemical formula 2 and chemical formula 3.

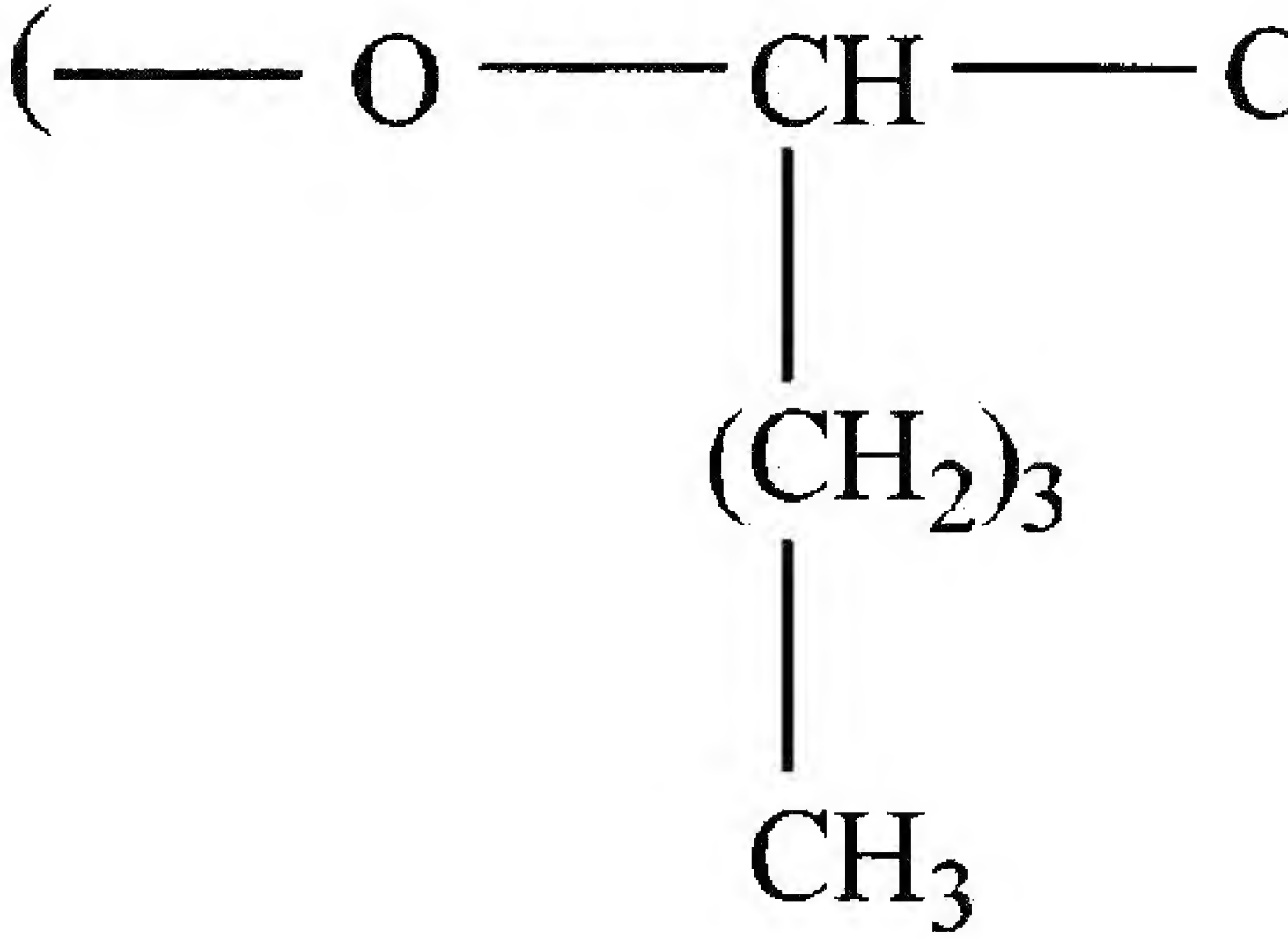
[Chemical formula 1]



[Chemical formula 2]



[Chemical formula 3]

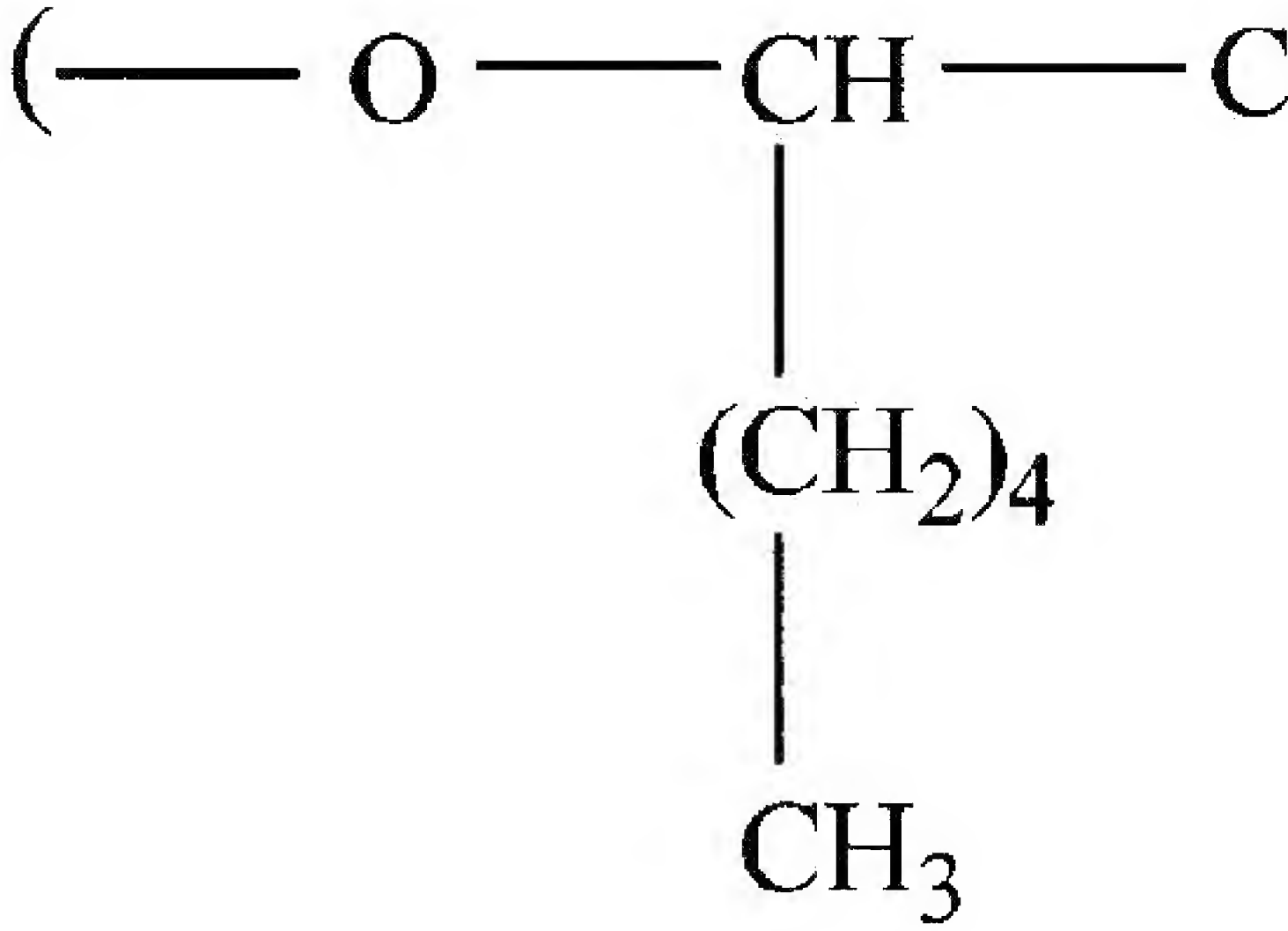


A of the chemical formula 1 0~90% , and the b of the chemical formula 2 are 0~90%. The c of the chemical formula 3 is 10~95%.

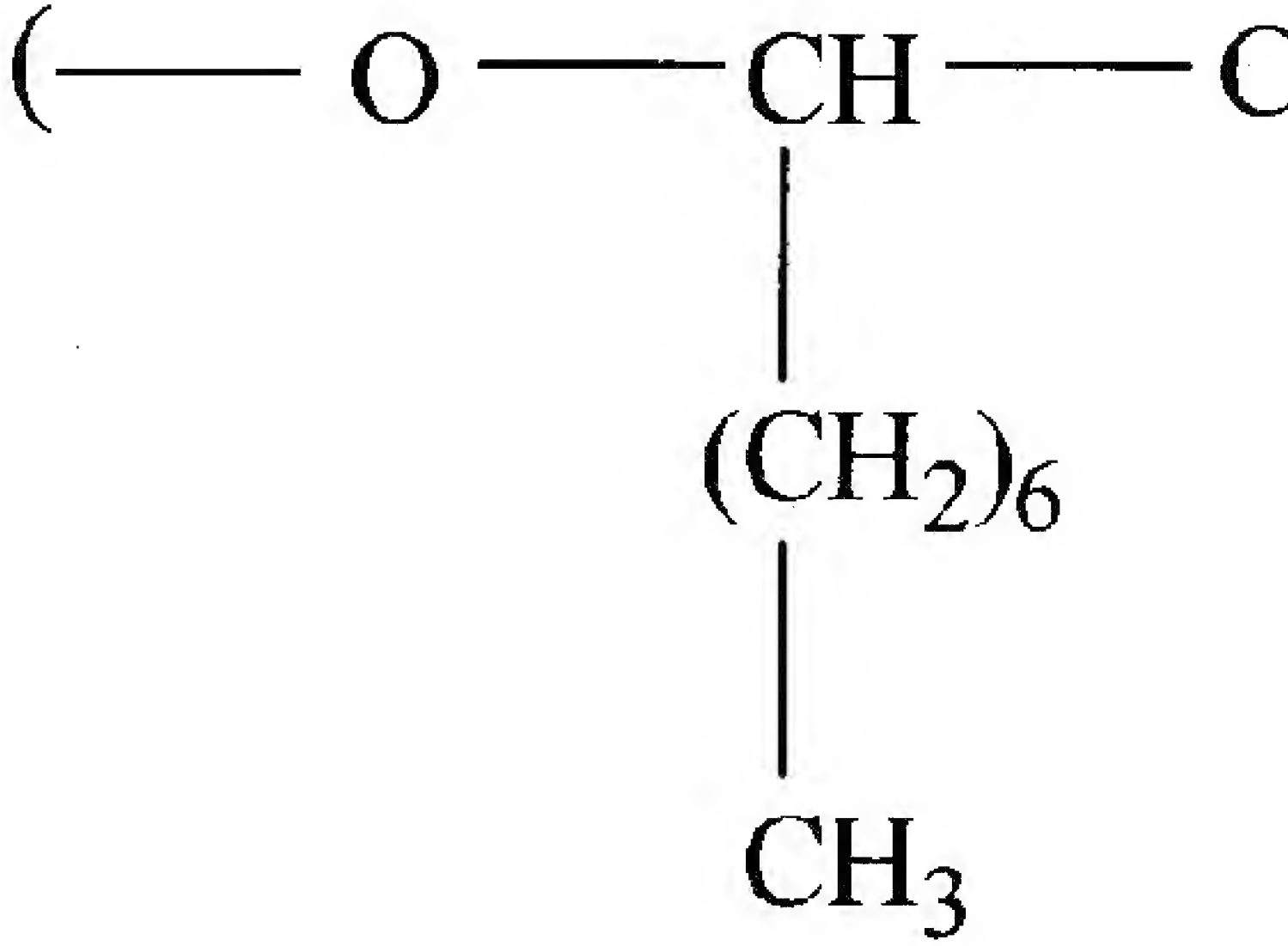
Claim 7 :

The polyhydroxyalkanoate copolymer of claim 5, wherein monomer is 3- hydroxyoctanoate of the estival chemical formula 4, 3- hydroxy dodecanoate of 3- hydroxy decanoate of the chemical formula 5 and chemical formula 6.

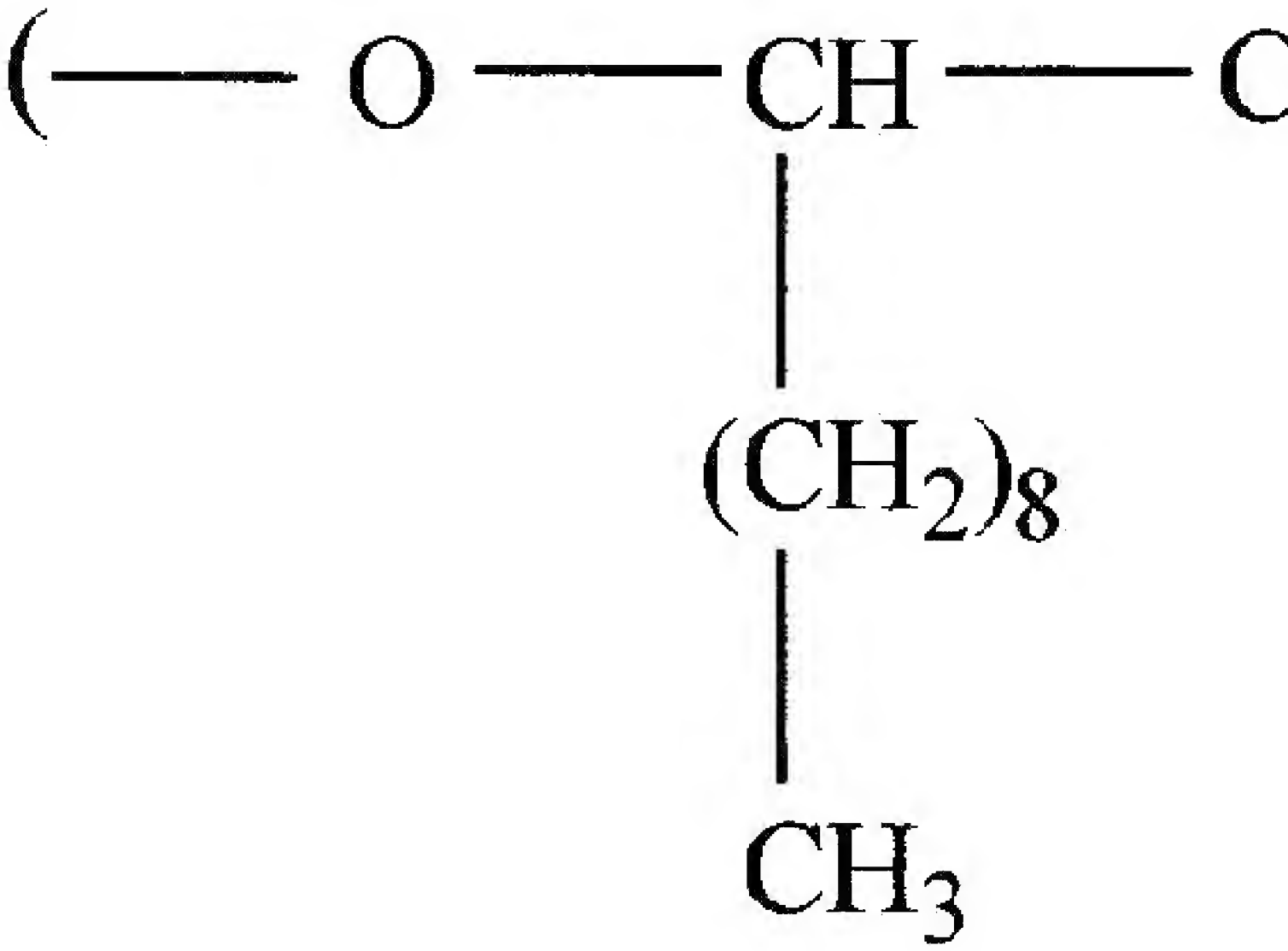
[Chemical formula 4]



[Chemical formula 5]



[Chemical formula 6]

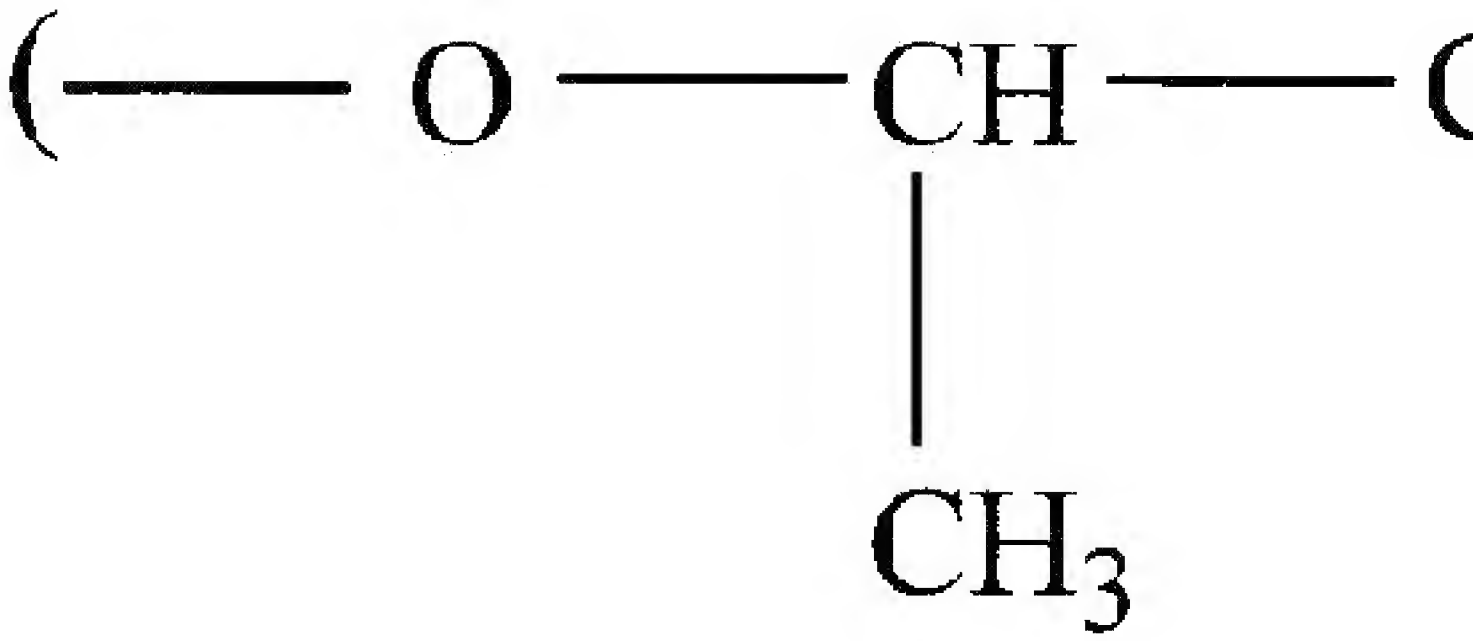


The d of the chemical formula 4 described in the above 0~55% , and the e of the chemical formula 5 are 45~90%. The f of the chemical formula 6 is 0~55%.

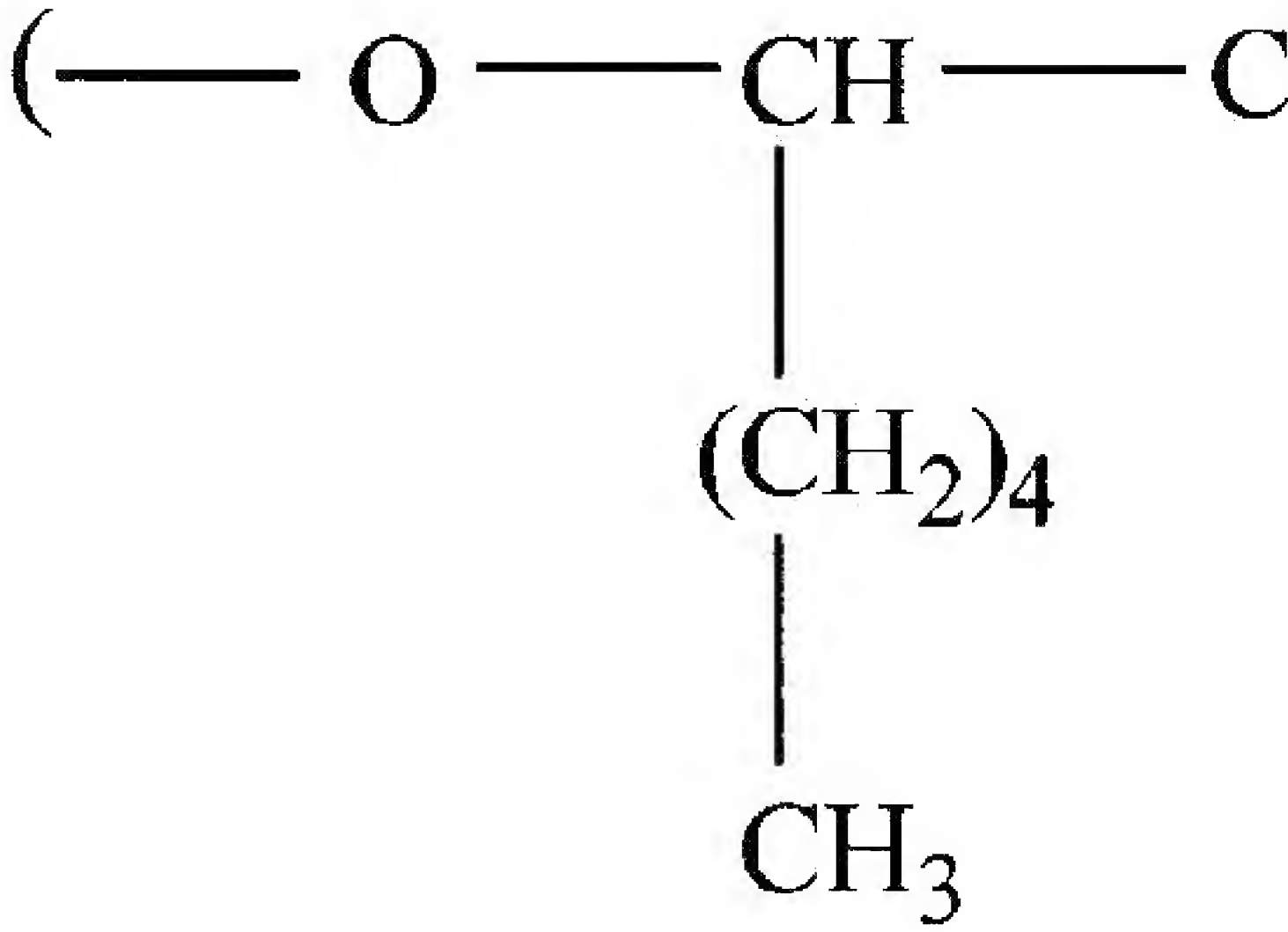
Claim 8 :

The polyhydroxyalkanoate copolymer of claim 5, wherein monomer is 3- hydroxybutyrate of the estival chemical formula 1, 3- hydroxyoctanoate of the chemical formula 4, 3- hydroxy dodecanoate of 3- hydroxy decanoate of the chemical formula 5 and chemical formula 6.

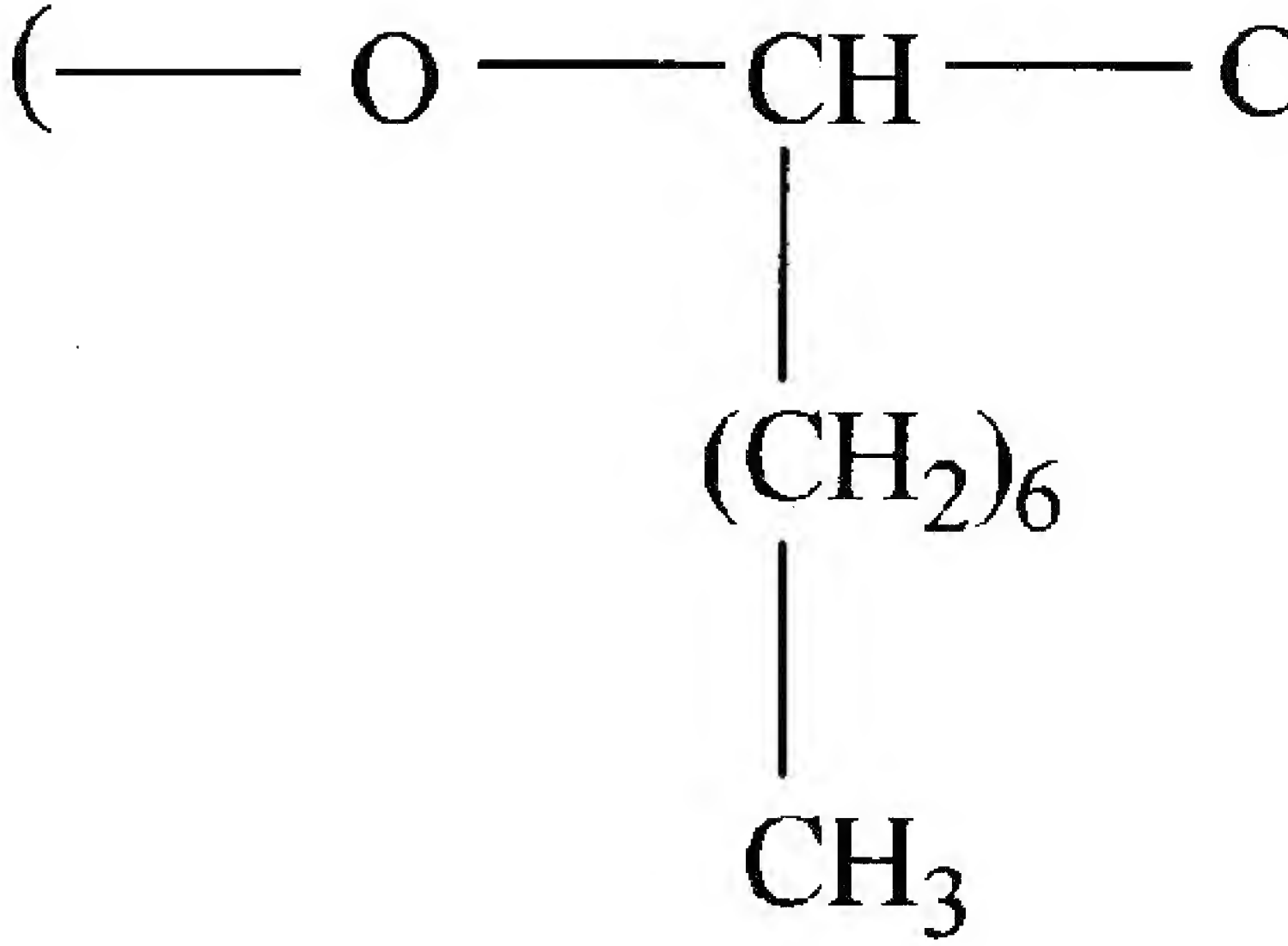
[Chemical formula 1]



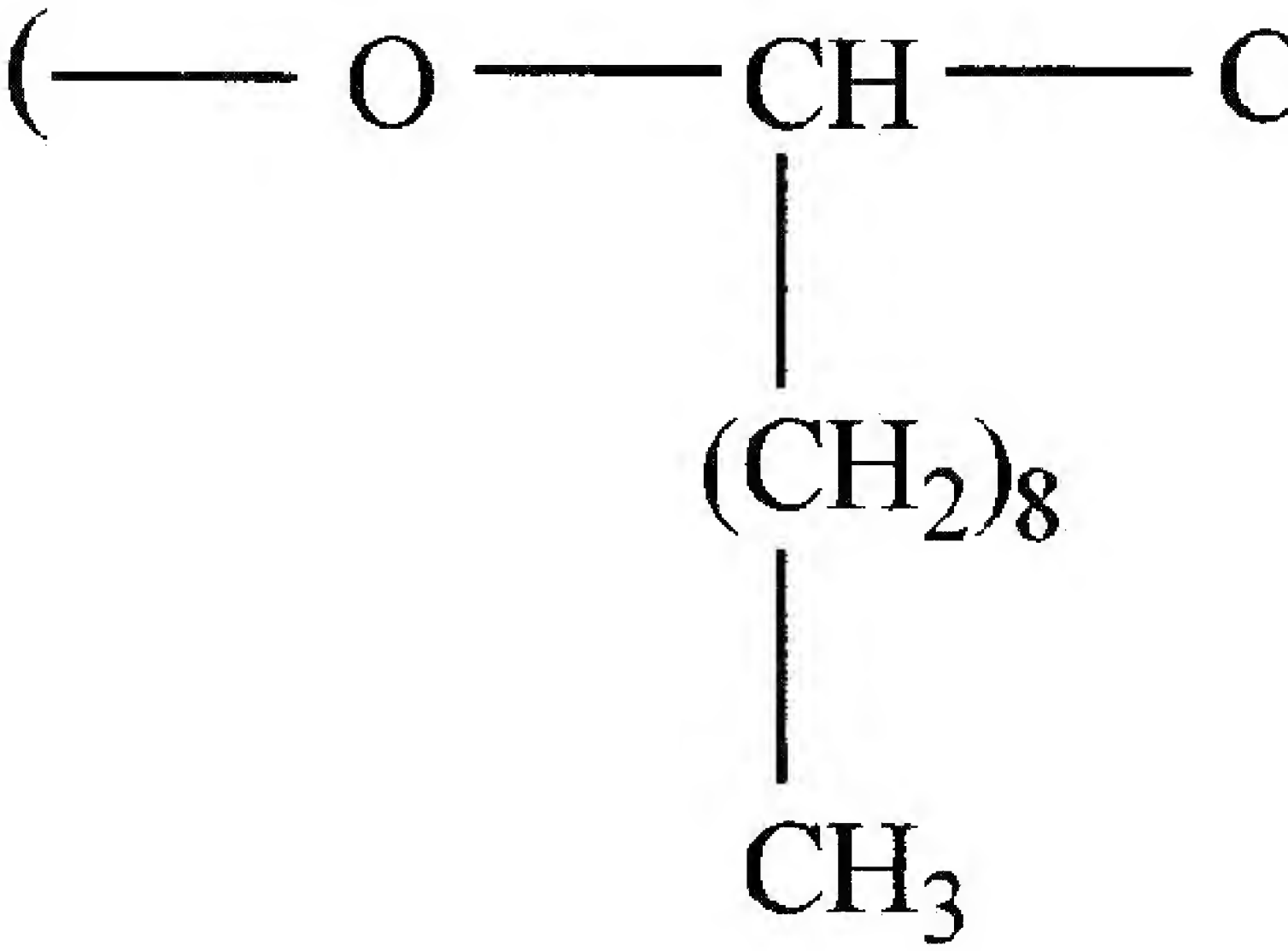
[Chemical formula 4]



[Chemical formula 5]



[Chemical formula 6]

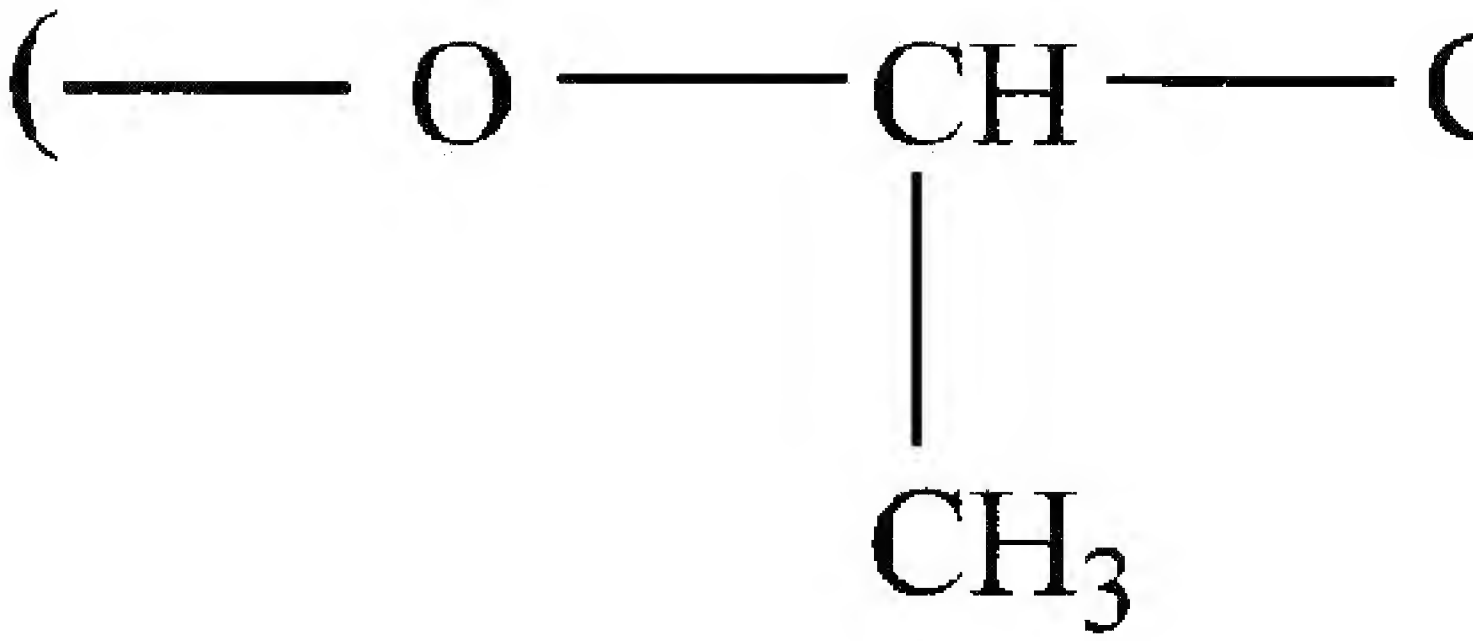


A of the chemical formula 1 described in the above 5~85% , and the d of the chemical formula 4 5~85% , and the e of the chemical formula 5 are 5~85%. The f of the chemical formula 6 is 5~85%.

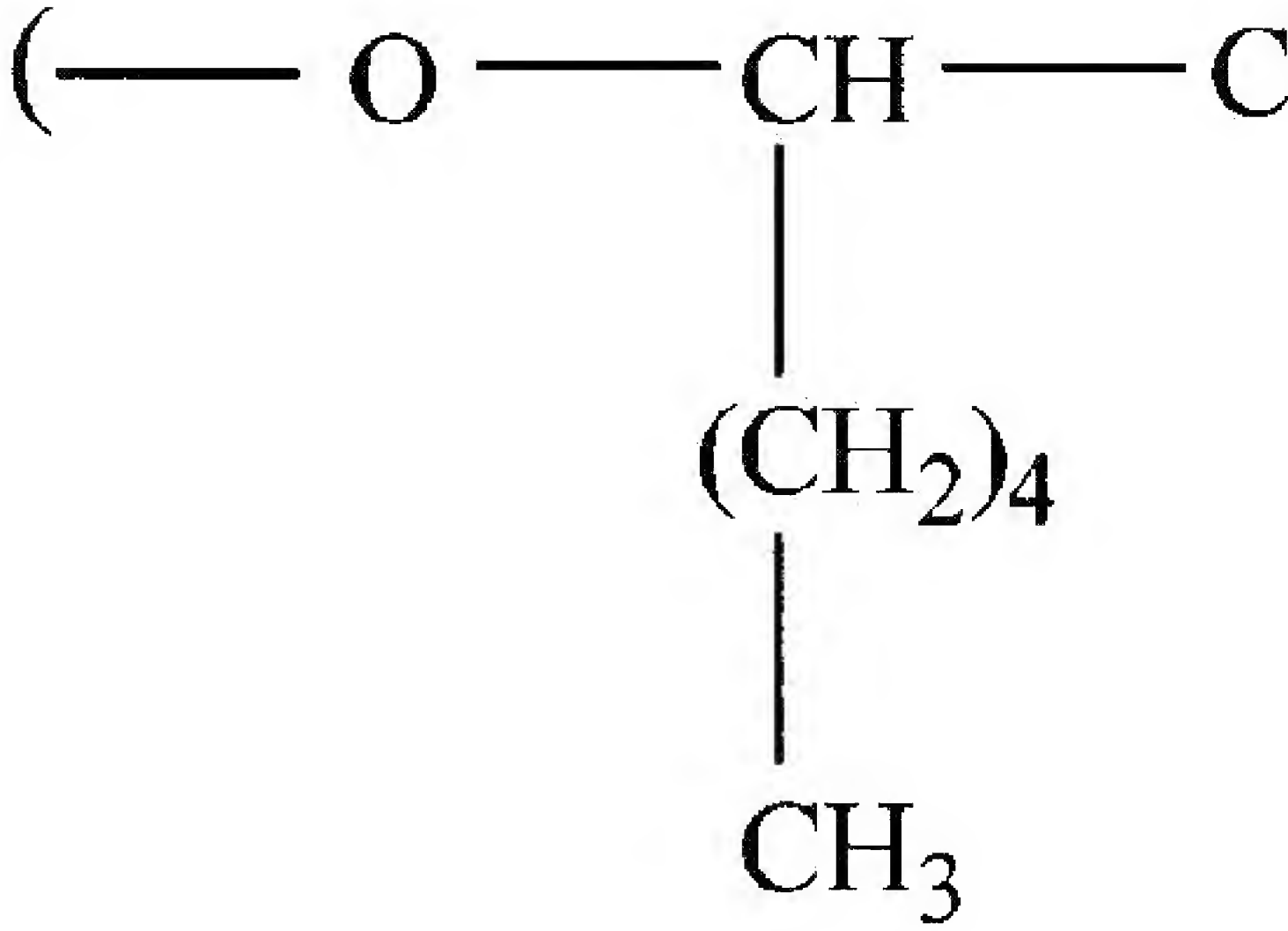
Claim 9 :

The polyhydroxyalkanoate copolymer of claim 5, wherein monomer is 3- hydroxybutyrate of the estival chemical formula 1, 3- hydroxy dodecanoate of 3- hydroxyoctanoate of the chemical formula 4 and chemical formula 6.

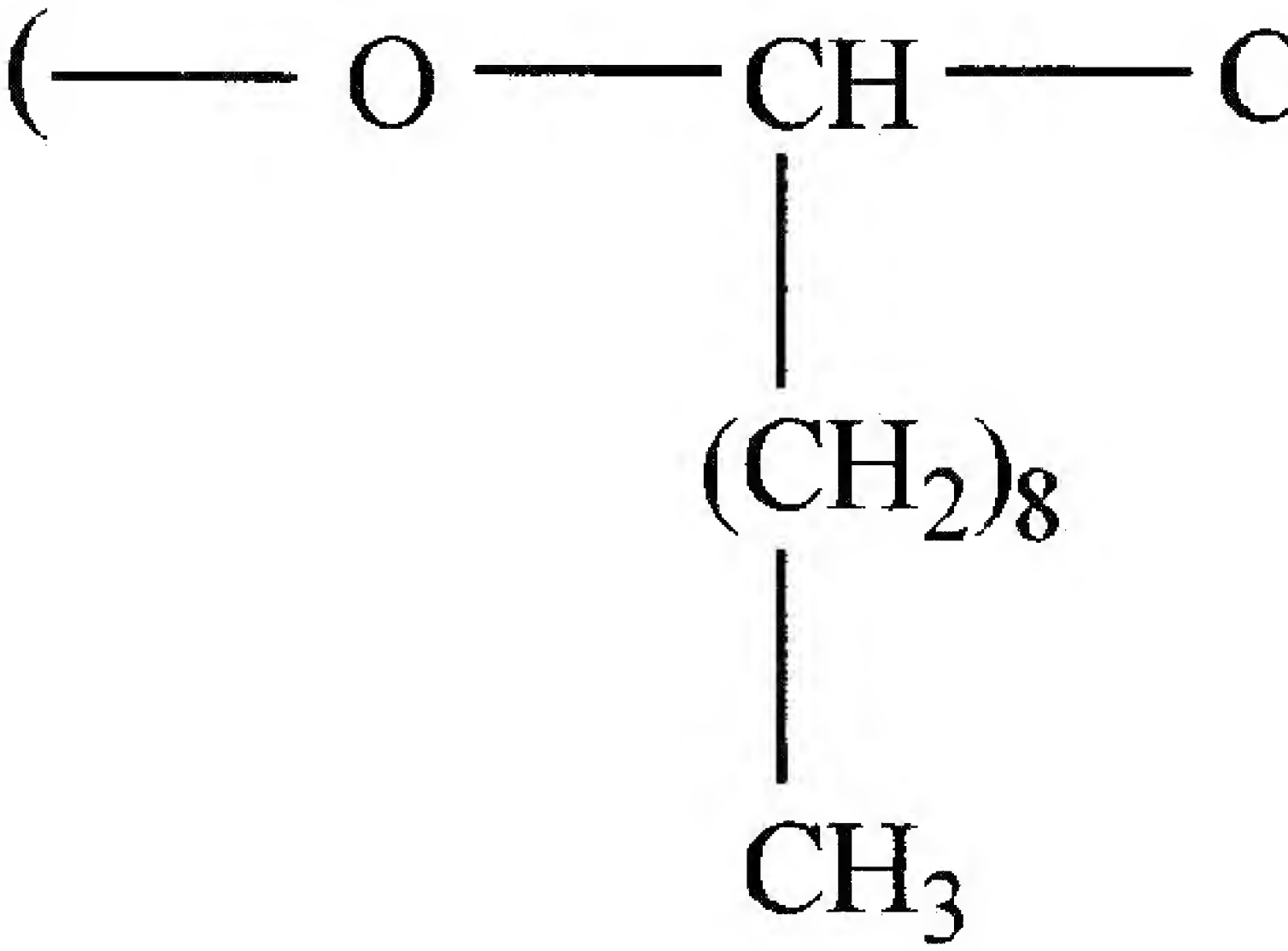
[Chemical formula 1]



[Chemical formula 5]



[Chemical formula 6]



A of the chemical formula 1 described in the above 5~90% , and the d of the chemical formula 4 the f of 5~90% and chemical formula 6 is 5~90%.

Claim 10 :
The manufacturing method of polyhydroxyalkanoate including the process of cultivating and producing polyhydroxyalkanoate in culture medium including the unsaturated carboxylic acid and/or the saturation the Pseudomonas sp. HJ-2 strain of claim 1.

Claim 11 :
The manufacturing method of the polyhydroxyalkanoate selected from the group of claim 10, wherein the unsaturated carboxylic acid and/or the saturation is made of the valerate, heptanoate, the vegetable oil, the animal fat and fish gasoline.

Claim 12 :
The elastic body including the polyhydroxyalkanoate copolymer of claim 5 to the main component.

Claim 13 :

The manufacturing method of the polyhydroxyalkanoate copolymer having the improved pull value including the process of stretching the film which it manufactures it uses the polyhydroxyalkanoate copolymer to one side direction or bidirectional in the crystallization former or after of claim 5.

Claim 14 :

The moldings wherein it is the fiber including the polyhydroxyalkanoate copolymer of claim 5, and fabric or film-type.

Drawings

Fig. 1

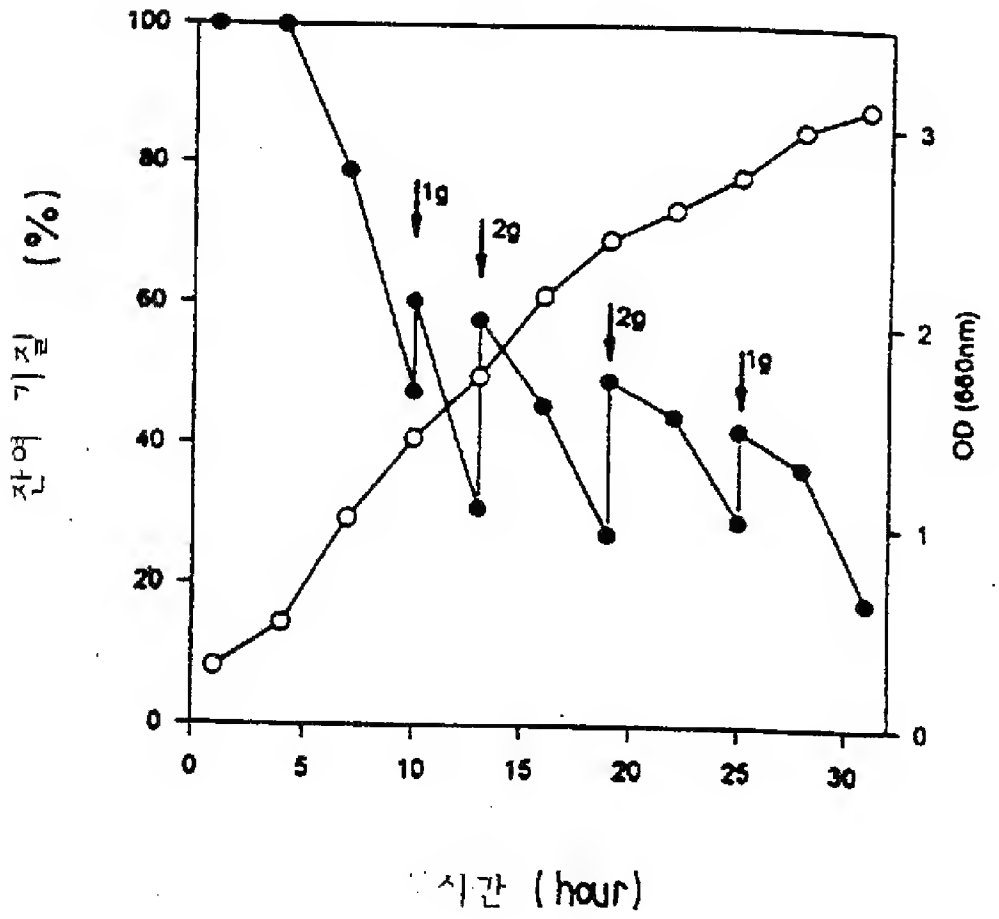


Fig. 2

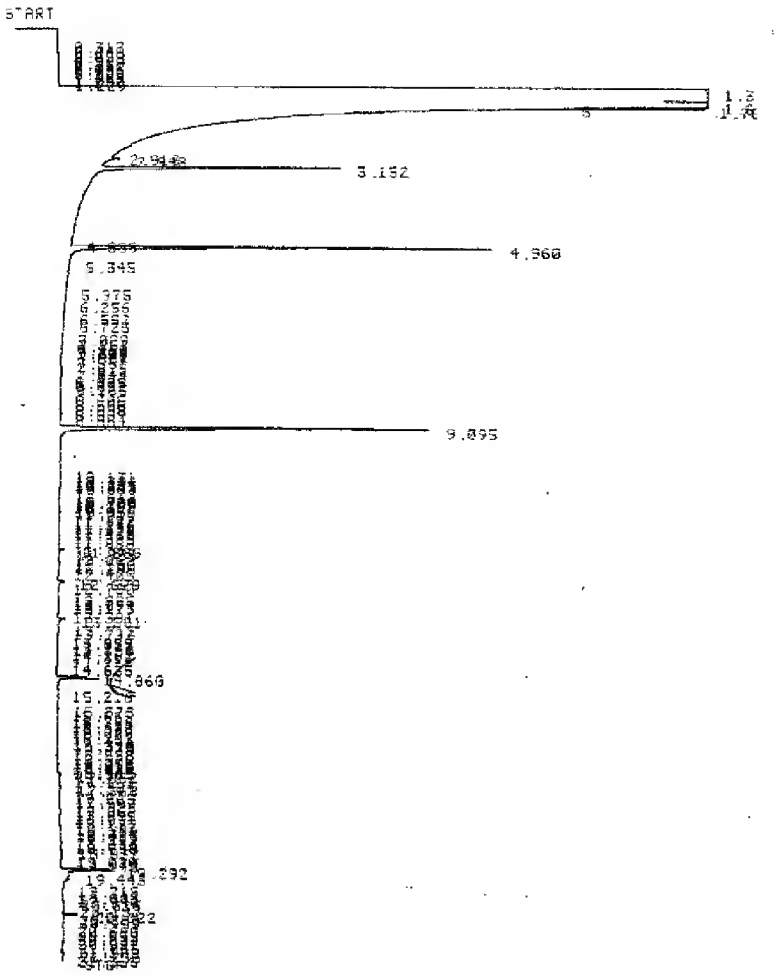


Fig. 3

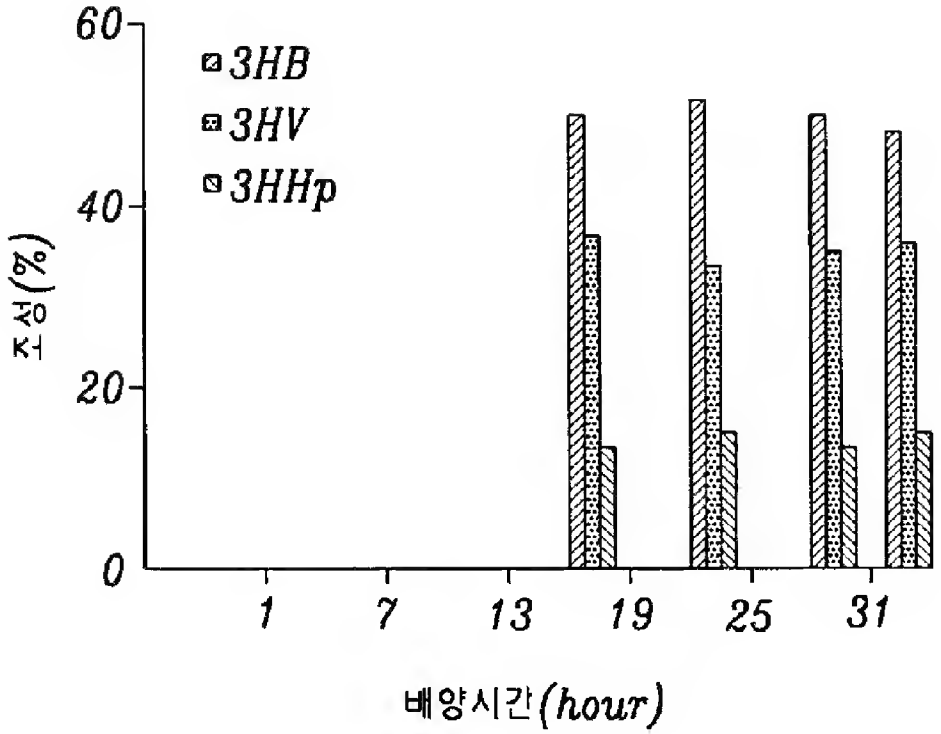


Fig. 4